(54) Title: HERBICIDE RESISTANT PLANTS

(57) Abstract: The present invention provides, inter alia, a glyphosate resistant EPSPS enzyme wherein in comparison with the wild type enzyme the EPSPS protein sequence is modified in that a first position is mutated so that the residue at this position is Ile rather that Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the conserved region GNAGTAMRPL in the wild type enzyme such that modified sequence reads GNAGIAMRSL. The invention also includes glyphosate resistant plants regenerated from material transformed with polynucleotides which encode such EPSPS enzymes and a method of selectively controlling weeds in a field comprising such plants and glyphosate sensitive weeds, by the application to the field of glyphosate or an agronomically acceptable derivative.

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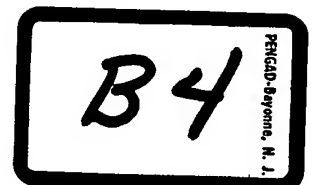
HERBICIDE RESISTANT PLANTS

The present invention relates to recombinant DNA technology, and in particular to the production of transgenic plants which exhibit substantial resistance or substantial tolerance to herbicides when compared with non transgenic like plants. The invention also

5   relates, *inter alia*, to the nucleotide sequences (and expression products thereof), which are used in the production of, or are produced by, the said transgenic plants.

Plants which are substantially "tolerant" to a herbicide when they are subjected to it provide a dose/response curve which is shifted to the right when compared with that provided by similarly subjected non tolerant like plants. Such dose/response curves have

10 "dose" plotted on the x-axis and "percentage kill","herbicidal effect" etc. plotted on the y-axis. Tolerant plants will typically require at least twice as much herbicide as non tolerant like plants in order to produce a given herbicidal effect. Plants which are substantially "resistant" to the herbicide exhibit few, if any, necrotic, lytic, chlorotic or other lesions when subjected to the herbicide at concentrations and rates which are typically employed by the

15 agricultural community to kill weeds in the field in which crops are to be grown for commercial purposes.

It is particularly preferred that the plants are substantially resistant or substantially tolerant to herbicides (hereinafter "glyphosate") which have 5-enol pyruvyl shikimate phosphate synthetase (hereinafter "EPSPS") as their site of action, of which N-

20 phosphonomethylglycine (and its various salts) is the pre-eminent example.

The herbicide may be applied either pre- or post emergence in accordance with usual techniques for herbicide application to fields comprising crops which have been rendered resistant to the herbicide. The present invention provides, *inter alia*, nucleotide sequences useful in the production of such herbicide tolerant or resistant plants.

25 According to the present invention there is provided a glyphosate resistant EPSPS enzyme wherein in comparison with the wild type EPSPS the protein sequence is modified in that a first position is mutated so that the residue at this position is Ile rather than Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the conserved region

30 GNAGTAMRPL in the wild type enzyme such that the modified sequence reads GNAGIAMRSL, wherein the enzyme does not comprise the sequence

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K(A/V)AKRAVVVGCGGKFPVE, characterised in that the enzyme is stable and possesses at least one of the following sequence motifs (i) to (viii) in which X is any amino acid and Z, Z_1 and Z_2 are any amino acid other than those specified:-

(i)   ALLMZAPLA, wherein Z is not S or T;

(ii)   EIEIZDKL, wherein Z is not V;

(iii)   FG(V/I)(K/S)ZEH, wherein Z is not V;

(iv)   AL(K/R)ZLGL, wherein Z is not R;

(v)   GLXVEZ$_1$DXZ$_2$XXXA(I/V)V, wherein Z_1 is not T and/or Z_2 is not E;

(vi)   ITPPZ$_1$K(L/V)(K/N)Z$_2$, wherein Z_1 is not K and/or Z_2 is not T;

(vii)   TIZ(D/N)PGCT, wherein Z is not N or L;

(viii) (D/N)YFXVLXZXX(K/R)H, wherein Z is not R.

A preferred embodiment of the enzyme comprises at least two of the motifs (i), (ii), (iii), (v) and (vi). In specific embodiments of the present inventive enzyme in motif (i) Z is A; in motif (ii) Z is I; in motif (iii) Z is A; in motif (iv) Z is K, T or A; in motif (v) Z_1 is R or A or less preferably D or E and Z_2 is preferably V or A or less preferably T; in sequence motif (vi) Z_1 is E or A and Z_2 is P, I or V; in motif (vii) Z is R or K; and in motif (viii) Z is T or S or less preferably Q. Particularly preferred embodiments of the said enzyme comprise one or more sequences selected from the group consiting of:

(i)   (I/V)VEGCGG(I/L/Q)FP(V/A/T)(S/G)

(ii)   (I/V)VVGCGG(I/L/Q)FP(V/A/T)E;

(iii)   (I/V)VVGCGG(I/L/Q)FP(V/A/T)(S/G);

(iv)   (I/V)VVGCGGKFP(V/A/T)(S/G);

(v)   (I/V)VEGCGGKFP(V/A/T)E;

(vi)   (I/V)VEGCGG(I/L/Q)FP(V/A/T)E;

(vii)   (I/V)VEGCGGKFP(V/A/T)(S/G).

The present invention also comprises an isolated polynucleotide comprising a region which encodes the present inventive enzyme, particular such polynucleotides comprising , for example, a sequence depicted in SEQ ID Nos. 4 or 5, or a sequence obtained by hybridising an intron located in the SEQ ID 4 or 5 sequences with polynucleotides comprised in plant genomic libraries. The invention also includes an isolated polynucleotide comprising a region encoding a chloroplast transit peptide and a glyphosate resistant

dicotyledenous 5-enolpyruvylshikimate phosphate synthase (EPSPS) 3' of the peptide, the said region being under expression control of a plant operable promoter, with the *provisos* that the said promoter is not heterologous with respect to the said region, and the chloroplast transit peptide is not heterologous with respect to the said synthase.

5   By "heterologous" is meant from a different source, and correspondingly "non-heterologous" means derived from the same source - but at a gene rather than organism or tissue level. For example the CaMV35S promoter is clearly heterologous with respect to a petunia EPSPS coding sequence insofar as the promoter is derived from a virus and the sequence - the expression of which it controls - from a plant. The term "heterologous"

10   according to the present invention has a still narrower meaning, however. For example "heterologous" as it relates to the present invention means that the petunia EPSPS coding sequence is "heterologous" with respect to, for example, a promoter also derived from petunia - other than that which controls expression of the EPSPS gene. In this sense the petunia promoter derived from the petunia EPSPS gene then used to control expression of an

15   EPSPS coding sequence likewise-derived from petunia is "non-heterologous" with respect to the said coding sequence. "Non-heterologous" does not mean, however, that the promoter and coding sequence must necessarily have been obtained from one and the same (original or progenitor) polynucleotide. Likewise with respect to transit peptides. For example, a rubisco chloroplast transit peptide derived from sunflower is "heterologous" with respect to the

20   coding sequence of an EPSPS gene likewise derived from sunflower (the same plant, tissue or cell). A rubisco transit peptide encoding sequence derived from sunflower is "non-heterologous" with respect to a rubisco enzyme encoding-sequence also derived from sunflower even if the origins of both sequences are different polynucleotides which may have been present in different cells, tissues or sunflower plants.

25   Most particularly preferred forms of the said polynucleotide include a construct which comprises the following components in the 5' to 3' direction of transcription: either (1):-

(i)   At least one transcriptional enhancer being that enhancing region which is upstream from the transcriptional start of the sequence from which the enhancer is obtained and which enhancer *per se* does not function as a promoter either in the

30   sequence in which it is endogenously comprised or when present heterologously as part of a construct;

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(ii)   The promoter from the soybean EPSPS gene;

(iii)   The soybean genomic sequence which encodes the soybean EPSPS
chloroplast transit peptide;

(iv)   The genomic sequence which encodes the soybean EPSPS;

(v)   A transcriptional terminator;

wherein the soybean EPSPS coding sequence is modified in comparison with a wild type
sequence in that a first position is mutated so that the residue at this position is Ile rather than
Thr and a second position is mutated so that the residue at this position is Ser rather than Pro,
the mutations being introduced into EPSPS sequences which comprise the conserved region
GNAGTAMRPLTAAV in the wild type enzyme such that modified sequence reads
GNAGIAMRSLTAAV; or (2)

(i)   At least one transcriptional enhancer being that enhancing region which is
upstream from the transcriptional start of the sequence from which the enhancer is
obtained and which enhancer *per se* does not function as a promoter either in the
sequence in which it is endogenously comprised or when present heterologously as
part of a construct;

(ii)   The promoter from the *Brasicca napus* EPSPS gene;

(iii)   The *Brasicca napus* genomic sequence which encodes the *Brasicca napus*
EPSPS chloroplast transit peptide;

(iv)   The genomic sequence which encodes the *Brasicca napus* EPSPS;

(v)   A transcriptional terminator;

wherein the *Brasicca napus* EPSPS coding sequence is modified in comparison with a wild
type sequence in that a first position is mutated so that the residue at this position is Ile rather
than Thr and a second position is mutated so that the residue at this position is Ser rather than
Pro, the mutations being introduced into EPSPS sequences which comprise the conserved
region GNAGTAMRPLTAAV in the wild type enzyme such that the modified sequence
reads GNAGIAMRSLTAAV.

The present inventive polynucleotide, particularly the two disclosed in the
immediately preceding paragraphs, may further comprise a sequence which encodes a
chloroplast transit peptide/phosphoenolpyruvate synthase (CTP/PPS) or a chloroplast transit
peptide/pyruvate orthophosphate di-kinase (CTP/PPDK), which sequence is under

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expression control of a plant operable promoter. It is particularly preferred that the PPDK protein sequence is directed to the chloroplast *in planta* via its autologous transit peptide, and accordingly the polynucleotide of the immediately preceding sentence encodes such a non-heterologous combination. The PPDK encoding sequence can be derived from either a monocot or a dicot and the protein thus encoded is optionally not cold-labile. By "not cold labile" is meant that the enzyme retains at least 50% of its activity when incubated at 0 degrees Celsius for 5 minutes under experimental conditions similar to those referred to in Usami *et al* (1995) Plant Mol. Biol., 27, 969-980. Such a 'cold stable' enzyme is at least two-fold more stable than the like enzyme isolated from *F. bidentis*. The PPS or PPDK enzymes provide for elevated levels of PEP in the chloroplast which is the site of action of glyphosate and which contains the EPSPS enzyme.

The enhancing region of the polynucleotide preferably constitutes a sequence the 3' end of which is at least 40 nucleotides upstream of the closest transcriptional start of the sequence from which the enhancer is obtained. In a further embodiment of the polynucleotide, the enhancing region constitutes a region the 3' end of which is at least 60 nucleotides upstream of the said closest start, and in a still further embodiment of the polynucleotide the said enhancing region constitutes a sequence the 3' of which is at least 10 nucleotides upstream from the first nucleotide of the TATA consensus of the sequence from which the enhancer is obtained.

The polynucleotide according to the invention may comprise two or more transcriptional enhancers, which in a particular embodiment of the polynucleotide may be tandemly present.

In the present inventive polynucleotide the 3' end of the enhancer, or first enhancer if there is more than one present, may be between about 100 to about 1000 nucleotides upstream of the codon corresponding to the translational start of the EPSPS transit peptide or the first nucleotide of an intron or other untranslated leader sequence in the 5' untranslated region in the case that the said region contains an intron or other untranslated sequence. In a more preferred embodiment of the polynucleotide, the 3' end of the enhancer, or first enhancer, is between about 150 to about 1000 nucleotides upstream of the codon corresponding to the translational start of the EPSPS transit peptide or the first nucleotide of in the 5' untranslated region, and in a still more preferred embodiment the 3' end of the

enhancer, or first enhancer, may be between about 300 to about 950 nucleotides upstream of the codon corresponding to the translational start of the EPSPS transit peptide or the first nucleotide of an intron or other untranslated leader sequence in the 5' untranslated region. In a yet more preferred embodiment, the 3' end of the enhancer, or first enhancer, may be
5   located between about 770 and about 790 nucleotides upstream of the codon corresponding to the translational start of the EPSPS transit peptide or the first nucleotide of an intron or other untranslated leader sequence in the 5' untranslated region.

In an alternative inventive polynucleotide, the 3' end of the enhancer, or first enhancer, may be located between about 300 to about 380 nucleotides upstream of the codon
10   corresponding to the translational start of the EPSPS transit peptide or the first nucleotide of an intron or other untranslated leader sequence in the 5' untranslated region, and in a preferred embodiment of this alternative polynucleotide the 3' end of the enhancer, or first enhancer, is located between about 320 to about 350 nucleotides upstream of the codon corresponding to the translational start of the EPSPS transit peptide, or the first nucleotide of
15   an intron or other untranslated leader sequence in the 5' untranslated region.

In the polynucleotide according to the invention, the region upstream of the promoter from the EPSPS gene may comprise at least one enhancer derived from a sequence which is upstream from the transcriptional start of a promoter selected from the group consisting of those of the actin, rolDFd, S-adenosyl homocysteinase, histone, tubulin, polyubiquitin and
20   plastocyanin genes and the CaMV35S and FMV35S genes.

Accordingly the present inventive polynucleotide may comprise in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region
25   derived from a sequence which is upstream from the transcriptional start of an actin gene.

Alternatively, the polynucleotide may comprise in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region derived from a sequence
30   which is upstream from the transcriptional start of the rolDfd gene.

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Alternatively, the polynucleotide may comprise in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region derived from a sequence
5   which is upstream from the transcriptional start of a histone gene.

Alternatively, the polynucleotide may comprise in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region derived from a sequence
10   which is upstream from the transcriptional start of a tubulin gene.

Alternatively, the polynucleotide may comprise in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of the FMV35S promoter and a second enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream
15   from the transcriptional start of the CaMV35S promoter.

Alternatively, the polynucleotide may comprise in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of the CaMV35S promoter and a second enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream
20   from the transcriptional start of the FMV35S promoter.

Whatever the identity and juxtaposition of the various enhancers present in the polynucleotide, the nucleotides 5' of the codon which constitutes the translational start of the EPSPS chloroplast transit peptide may be Kozack preferred. What is meant by this is well known to the skilled man and will be further apparent from the examples below.

25   Particularly preferred embodiments of the present inventive polynucleotide comprise up stream of the sequence encoding the EPSPS chloroplast transit peptide a sequence encoding a non-translated 5' leader sequence derived from a relatively highly expressed gene, such as that of the glucanase, chalcone synthase and Rubisco genes.

The polynucleotide of the invention may also comprise a virally derived translational
30   enhancer located within the non translated region 5' of the genomic sequence which encodes the EPSPS chloroplast transit peptide. The man skilled in the art is aware of the identity of

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such suitable translational enhancers - such as the Omega and Omega prime sequences derived from TMV and that derived from the tobacco etch virus, and how such translational enhancers can be introduced into the polynucleotide so as to provide for the desired result of increased protein expression.

5   The polynucleotide according to the invention may further comprise regions encoding proteins capable of conferring upon plant material containing it at least one of the following agronomically desirable traits: resistance to insects, fungi, viruses, bacteria, nematodes, stress, dessication, and herbicides. Whilst such a polynucleotide contemplates the herbicide resistance conferring gene being other than an EPSPS, such as glyphosate oxido-reductase

10   (GOX) for example, the herbicide may be other than glyphosate in which case the resistance conferring genes may be selected from the group encoding the following proteins: phosphinothricin acetyl transferase (PAT), hydroxyphenyl pyruvate dioxygenase (HPPD), glutathione S transferase (GST), cytochrome P450, Acetyl-COA carboxylase (ACCase), Acetolactate synthase (ALS), protoporphyrinogen oxidase (PPO), dihydropteroate synthase,

15   polyamine transport proteins, superoxide dismutase (SOD), bromoxynil nitrilase, phytoene desaturase (PDS), the product of the *tfd*A gene obtainable from *Alcaligenes eutrophus*, and known mutagenised or otherwise modified variants of the said proteins.   In the case that the polynucleotide provides for multiple herbicide resistance such herbicides may be selected from the group consisting of a dinitroaniline herbicide, triazolo-pyrimidines, a uracil, a

20   phenylurea, a triketone, an isoxazole, an acetanilide, an oxadiazole, a triazinone, a sulfonanilide, an amide, an anilide, an isoxaflutole, a flurochloridone, a norflurazon, and a triazolinone type herbicide and the post-emergence herbicide is selected from the group consisting of glyphosate and salts thereof, glufosinate, asulam, bentazon, bialaphos, bromacil, sethoxydim or another cyclohexanedione, dicamba, fosamine, flupoxam, phenoxy propionate,

25   quizalofop or another aryloxy-phenoxypropanoate, picloram, fluormetron, atrazine or another triazine, metribuzin, chlorimuron, chlorsulfuron, flumetsulam, halosulfuron, sulfometron, imazaquin, imazethapyr, isoxaben, imazamox, metosulam, pyrithrobac, rimsulfuron, bensulfuron, nicosulfuron, fomesafen, fluroglycofen, KIH9201, ET751, carfentrazone, mesotrione, sulcotrione, paraquat, diquat, bromoxynil and fenoxaprop.

30   A particular embodiment of the polynucleotide according to the invention provides for resistance to both glyphosate and a protoporphyrinogen oxidase (PPGO) inhibitor, in

particular butafenicil, for which the corresponding resistance protein is a PPGO or an inhibitor resistant variant thereof. A particularly preferred construct encoding such a herbicide resistance conferring combination comprises the EPSPS encoding sequence of the invention in combination with a sequence which encodes a PPDK or PPS in combination

5   with a PPGO inhibitor resistant PPGO enzyme encoding sequence – such sequences being directed to the chloroplast – if desirable – by their autologous transit peptides and being under the expression control of non-heterologous promoters if the protein encoding sequences are of plant as opposed to bacterial origin.

       In the case that the polynucleotide comprises sequences encoding insecticidal

10   proteins, these proteins may be selected from the group consisting of crystal toxins derived from Bt, including secreted Bt toxins; protease inhibitors, lectins, Xenhorabdus/Photorhabdus toxins. The fungus resistance conferring genes may be selected from the group consisting of those encoding known AFPs, defensins, chitinases, glucanases, and Avr-Cf9. Particularly preferred insecticidal proteins are crylAc, crylAb, cry3A, Vip

15   1A,Vip 1B, Vip3A, Vip3B, cysteine protease inhibitors, and snowdrop lectin. In the case that the polynucleotide comprises bacterial resistance conferring genes these may be selected from the group consisting of those encoding cecropins and techyplesin and analogues thereof. Virus resistance conferring genes may be selected from the group consisting of those encoding virus coat proteins, movement proteins, viral replicases, and anti-sense and

20   ribozyme sequences which are known to provide for virus resistance; whereas the stress, salt, and drought resistance conferring genes may be selected from those that encode Glutathione-S-transferase and peroxidase, the sequence which constitutes the known CBF1 regulatory sequence and genes which are known to provide for accumulation of trehalose.

       The polynucleotide according to the invention may be modified to enhance

25   expression of the protein encoding sequences comprised by it, in that mRNA instability motifs and/or fortuitous splice regions may be removed, or crop preferred codons may be used so that expression of the thus modified polynucleotide in a plant yields substantially similar protein having a substantially similar activity/function to that obtained by expression of the protein encoding regions of the unmodified polynucleotide in the organism in which

30   such regions of the unmodified polynucleotide are endogenous. The degree of identity between the modified polynucleotide and a polynucleotide endogenously contained within

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the said plant and encoding substantially the same protein may be such as to prevent co-suppression between the modified and endogenous sequences. In this case the degree of identity between the sequences should preferably be less than about 70%.

The invention still further includes a biological or transformation vector comprising the present inventive polynucleotide. Accordingly, by "vector" is meant, *inter alia*, one of the following: a plasmid, virus, cosmid or a bacterium transformed or transfected so as to contain the polynucleotide.

The invention still further includes plant material which has been transformed with the said polynucleotide or vector, as well as such transformed plant material which has been, or is, further transformed with a polynucleotide comprising regions encoding proteins capable of conferring upon plant material containing it at least one of the following agronomically desirable traits: resistance to insects, fungi, viruses, bacteria, nematodes, stress, dessication, and herbicides.

The invention still further includes morphologically normal fertile whole plants which result from the crossing of plants which have been regenerated from material which has been transformed with the polynucleotide of the invention and plants which result from regeneration of material transformed with a polyncleotide which comprises a sequence which encodes a protein, such as a phosphoenolpyruvate synthase (PPS) or pyruvate orthophosphate di-kinase (PPDK) which proteins are capable of providing for elevated levels of phosphoenolpyruvate in the chloroplast. Such proteins are directed to the chloroplast by a suitable transit peptide – in the case of the PPDK enzyme preferably by the autologous peptide.

The invention still further includes morphologically normal fertile whole plants which contain the present inventive polynucleotide and which result from the crossing of plants which have been regenerated from material transformed with the present inventive polynucleotide or vector, and plants which have been transformed with a polynucleotide comprising regions encoding a CTP/PPS or CTP/PPDK, and/or regions encoding proteins capable of conferring upon plant material containing it at least one of the following agronomically desirable traits: resistance to insects, fungi, viruses, bacteria, nematodes, stress, dessication, and herbicides. The invention also includes progeny of the resultant plants, their seeds and parts.

Plants of the invention may be selected from the group consisting of field crops, fruits and vegetables such as canola, sunflower, tobacco, sugar beet, cotton, maize, wheat, barley, rice, sorghum, mangel worzels, tomato, mango, peach, apple, pear, strawberry, banana, melon, potato, carrot, lettuce, cabbage, onion, soya spp, sugar cane, pea, field beans, poplar, grape, citrus, alfalfa, rye, oats, turf and forage grasses, flax and oilseed rape, and nut producing plants insofar as they are not already specifically mentioned, their progeny, seeds and parts.

Particularly preferred such plants include soybean, canola, brassica, cotton, sugar beet, sunflower, peas, potatoes and mangel worzels.

The invention still further comprises a method of selectively controlling weeds in a field, the field comprising weeds and plants of the invention or the herbicide resistant progeny thereof, the method comprising application to the field of a glyphosate type herbicide in an amount sufficient to control the weeds without substantially affecting the plants. According to this method, one or more of a herbicide, insecticide, fungicide, nematicide, bacteriocide and an anti-viral may be applied to the field (and thus the plants contained within it) either before or after application of the glyphosate herbicide.

The invention still further provides a method of producing plants which are substantially tolerant or substantially resistant to glyphosate herbicide, comprising the steps of:

(i)   transforming plant material with the polynucleotide or vector of the invention;

(ii)   selecting the thus transformed material; and

(iii)   regenerating the thus selected material into morphologically normal fertile whole plants.

The transformation may involve the introduction of the polynucleotide into the material by any known means, but in particular by: (i) biolistic bombardment of the material with particles coated with the polynucleotide; (ii) by impalement of the material on silicon carbide fibres which are coated with a solution comprising the polynucleotide; or (iii) by introduction of the polynucleotide or vector into *Agrobacterium* and co-cultivation of the thus transformed *Agrobacterium* with plant material which is thereby transformed and is subsequently regenerated. Plant transformation, selection and regeneration techniques, which may require routine modification in respect of a particular plant species, are well

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known to the skilled man. The thus transformed plant material may be selected by its resistance to glyphosate.

The invention still further provides the use of the present inventive polynucleotide or vector in the production of plant tissues and/or morphologically normal fertile whole plants which are substantially tolerant or substantially resistant to glyphosate herbicide.

The invention still further includes a method of selecting biological material transformed so as to express a gene of interest, wherein the transformed material comprises the polynucleotide or vector of the invention, and wherein the selection comprises exposing the transformed material to glyphosate or a salt thereof, and selecting surviving material. The said material may be of plant origin, and may in particular be derived from a dicot selected from the group consisting of soybean, sugar beet, cotton and the *Brassicas*.

The invention still further includes a method for regenerating a fertile transformed plant to contain foreign DNA comprising the steps of:

(a)   producing regenerable tissue from said plant to be transformed;

(b)   transforming said regenerable tissue with said foreign DNA, wherein said foreign DNA comprises a selectable DNA sequence, wherein said sequence functions in a regenerable tissue as a selection device;

(c)   between about one day to about 60 days after step (b), placing said regenerable tissue from step (b) in a medium capable of producing shoots from said tissue, wherein said medium may further contain a compound used to select regenerable tissue containing said selectable DNA sequence to allow identification or selection of the transformed regenerated tissue;

(d)   after at least one shoot has formed from the selected tissue of step (c) transferring said shoot to a second medium capable of producing roots from said shoot to produce a plantlet, wherein the second medium optionally contains the said compound; and

(e)   growing said plantlet into a fertile transgenic plant wherein the foreign DNA is transmitted to progeny plants in Mendelian fashion, characterised in that the foreign DNA is, or the selectable DNA sequence comprised by the foreign DNA comprises, the polynucleotide according to the invention, and the said compound is glyphosate or a salt thereof. The plant may be a dicot as indicated above - more preferably selected from

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soybean, canola, brassica, cotton, sugar beet, sunflower, peas, potatoes and mangel worzel, and the said regenerable tissue may consist of embryogenic calli, somatic embryos, immature embryos etc.

The present invention also includes a diagnostic kit comprising means for detecting the present inventive enzyme or polynucleotide or enzymes encoded by it, and therefore suitable for identifying tissues or samples which contain these. The polynucleotides can be detected by PCR amplification as is known to the skilled man - based on primers which he can easily derive from the enzyme encoding sequences which are disclosed in this application. The enzymes *per se* can be detected by, for example, the use of antibodies which have been raised against them for diagnostically distinguishing the antigenic regions which they contain.

The present invention will be further apparent from the following description taken in conjunction with the associated drawings and sequence listings.

Of the Sequences:

SEQ ID No.1 depicts Brassicia napus genomic DNA.

2	Bn EPSPS5	(PCR primer)
3	Bn EPSPS3	(PCR primer)
4	BnF1	(PCR primer)
5	BnF2	(PCR primer)
6	BnF3	(PCR primer)
7	BnF4	(PCR primer)
8	BnF5	(PCR primer)
9	BnF6	(PCR primer)
10	BnF7	(PCR primer)
11	BnF8	(PCR primer)
12	BnF9	(PCR primer)
13	BnF10	(PCR primer)
14	BnF11	(PCR primer)
15	BnF12	(PCR primer)
16	BnXho	(PCR primer)
17	BnMUTBot	(PCR primer)

	18	BnMUTTop	(PCR primer)
	19	BnNde	(PRC primer)
	20	BnRBSEP	(PCR primer)
	21	Brasr 12	(PCR primer)
5	22	Brasr 11	(PCR Primer)
	23	Bnapus start rev	
	24	Genome walker 1	
	25	Genome walker 2	
	26	CaMV343	(PCR primer)
10	27	CaMV46C	(PCR primer)
	28	BnGUSXMa	(PCR primer)
	29	FMV enhancer	
	30	CaMV35S enhancer	
	31	Soybean Genomic EPSPS Sequence clone 1/14	
15	32	Soybean Genomic EPSPS Sequence clone 1/12	
	33	EPSPS 10 reverse	(PCR primer)
	34	EPSPS 4 forward	(PCR primer)
	35	SEQ ID No. 31 containing double mutation	
	36	SEQ ID No. 32 containing double mutation	
20	37	FMV35S90 enhancer	
	38	FMV35S46 enhancer	
	39	Gm Term Bam	(PCR primer)
	40	Gm Term Eco	(PCR primer)
	41	BnMUT2PST	(PCR primer)
25	42	BnMUTKPN	(PCR primer)
	43	GmRubPac	(PCR primer)
	44	GmRubbot	(PCR primer)
	45	GmRubtop	(PCR primer)
	46	GmRubKpn	(PCR primer)
30	47	ZMPPDK1	(PCR primer)
	48	ZMPPDK2	(PCR primer)

49	ECPPS1	(PCR primer)
50	ECPPS2	(PCR primer)
51-65	Motifs present in the stable EPSPS proteins of the invention	

5

EXAMPLES

Methods useful for cloning, expressing and characterising the stable glyphosate-resistant EPSP synthases of the current invention are described herein

Stable glyphosate- resistant EPSPSs of the current invention are characterised as

10   being stable (retaining > ~ 80% of their catalytic activity) after being incubated in a suitable buffer at 37 C for at least 4 hours. As exemplified below, enzyme stability experiments can suitably be carried out using substantially purified, part purified or crude preparations of enzyme extracts (for example the latter are readily obtained as extracts of transgenic plants engineered to express the glyphosate-resistant EPSPS or, similarly, extracts of microbes

15   engineered to express a transgene encoding the glyphosate-resistant EPSPS). Ideally, glyphosate-resistant enzymes are tested for stability both in a purified form and then as purified protein spiked back into a crude plant extract so as to test them under more realistic *in planta* -like conditions. In such extracts, the enzyme activity due to glyphosate-resistant EPSPS can easily be discriminated from background susceptible endogenous EPSPS by

20   testing activity over a range of glyphosate concentrations. At a discriminating concentration of glyphosate (e.g 0.1 mM glyphosate in the presence of 0.1 mM PEP) virtually all of the measured activity will originate from the resistant enzyme

Example. 1 Method for identification of stable glyphosate-resistant EPSP synthases

In order to test the stability of a glyphosate-resistant EPSPS, a test solution of the

25   enzyme (prepared as described below in buffer at pH ~ 7.0-7.5 and in purified or part purified form) is heated to 37 C for 4-5h. The glyphosate-resistant activity of the heated enzyme is then compared with the activity of a) a sample similarly prepared but kept at ice temperature and b) a similar sample but freshly prepared.

Stable glyphosate resistant enzymes of the current invention are characterised by the

30   fact that, following incubation for 5 h at 37 C, they lose little, if any, of their original glyphosate-resistant catalytic activity (< 20% , preferably < 15% and more preferably < 10%)

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relative to suitable controls when assayed as described below. Preferably the stability experiment is carried out using substantially pure enzyme although a very similar result will normally be obtained using crude preparations, or when pure protein is spiked back into crude extract. Methods suitably used for assay and preparation of enzyme extracts are

5   further detailed below. The stability of the glyphosate-resistant EPSPS can suitably be assessed in a variety of other, essentially equivalent ways (for example by heating the enzyme at a slightly higher temperature for a shorter time or a lower temperature for a longer period...e.g at 25 C for 3 d).

Example 2. Method for assaying EPSPS activity and determination of kinetic constants.

10   Assays are carried out generally according to the radiochemical method of Padgette et al 1987 (Archives of Biochemistry and Biophysics, 258(2) 564-573) with K+ ions as the major species of cationic counterion. Assays in a total volume of 50μl, in 50mM Hepes(KOH) pH 7.0 at 25°C, contain purified enzyme or plant extract (see below) diluted appropriately in Hepes pH 7.0 containing 10% glycerol, and 5mM DTT, ^{14}C PEP either as variable substrate(

15   for kinetic determinations) or fixed at 100 or 250 μM and shikimate 3 Phosphate (K+ salt) at 0.75 or 2 mM as indicated. Optionally, for assays of crude plant extracts, assays also contain 5 mM KF and/or 0.1 mM ammonium molybdate. Assays are started with the addition of ^{14}C phosphoenolpyruvate (cyclohexylammonium+ salt) and stopped after 2-10 minutes (2 minutes is preferable) with the addition of 50μl of a solution of 1 part 1M acetic acid and 9

20   parts ethanol. After stopping, 20μl is loaded onto a synchropak AX100 (25cm x 4.6mm) column and chromatographed using isocratic elution with a 0.28M potassium phosphate pH 6.5 mobile phase flowing at 0.5 ml/min over 35 minutes. Under these conditions the retention times for PEP and EPSP are, depending on the individual columns, ~ 19 and 25 minutes respectively. A CP 525TR scintillation counter is connected to the end of the AX

25   100 column. It is fitted with a 0.5ml flow cell, and the flow rate of scintillant (Ultima Flo AP) is set at 1ml/min. Relative peak areas of PEP and EPSP are integrated to determine the percentage conversion of labelled PEP to EPSP. Apparent K_m and Vmax values are determined by least squares fit to a hyperbola with simple weighting using the Grafit 3.09b from Erithacus Software Ltd. Km values are generally ascertained using 8-9 concentrations

30   of variable substrate ranging from K_m / 2 - 10 K_m and triplicate points. Except where

specifically noted, data points are only included in the analysis where there is < 30%
conversion of substrate to EPSP.

Shikimate-3-Pi (S3P) is prepared as follows, To 7mls of 0.3M TAPS pH 8.5
containing 0.05M Shikimate, 0.0665M ATP (Na salt), 10mM KF, 5mM DTT, and 0.05M

5    $MgCl_2.6H_2O$, 75µl of a 77 unit (µmol min^{-1}) ml^{-1} solution of shikimate kinase is added. After
24hrs at room temperature, the reaction is stopped by brief heating to 95^{0}C. The reaction
solution is diluted 50 fold in 0.01M Tris HCl pH 9, and chromatographed by anion exchange
on Dowex 1 X 8 - 400, using a 0 - 0.34M LiCl$_2$ gradient. The S3P fractions are combined,
freeze dried, and then redissolved in 7mls distilled H$_2$O. 28mls of 0.1M Ba(CH$_3$COOH)$_2$ and

10   189mls of absolute ethanol are then added. This solution is left to stir overnight at 4^{0}C. The
resulting precipitate of tri-Barium S3P is collected and washed in 30mls of 67% ethanol. The
washed precipitate is then dissolved in ~ 30mls distilled H$_2$O. By adding K$_2$SO$_4$ the K$^+$ salt of
S3P is produced as required. Great care is taken to add a minimal excess of sulphate. The
BaSO$_4$ precipitate is removed and the supernatant containing the required salt of S3P freeze

15   dried. Each salt is weighed and analysed by proton NMR. S3P preparations so-prepared are
> 90% pure according to proton NMR and, according to their weights and integration of 31P
NMR, contain only low residues of potassium sulphate.

Example 3. Preparation of extracts of plant material suitable for EPSPS assay

Callus or plantlet material (0.5 -1.0 g) is ground to a fine frozen powder in a liquid

20   nitrogen-chilled mortar and pestle. This powder is taken up in an equal volume of a suitable
chilled extraction buffer (for example, 50 mM Hepes/ KOH buffer at pH 7.5 containing 1
mM EDTA, 3 mM DTT, 1.7 mM 'pefabloc' (serine protease inhibitor), 1.5 mM leupeptin,
1.5 mM pepstatin A, 10% v/v glycerol and 1% polyvinylpyrolidone), resuspended, mixed
and centrifuged in a chilled centrifuge to bring down debris. The supernatant is exchanged

25   down a chilled PD10 column of Sephadex G25 into 25 mM Hepes/ KOH buffer at pH 7.5
containing 1 mM EDTA, 3 mM DTT and 10% v/v glycerol. Protein is estimated by the
Bradford method standardised using bovine serum albumen. A portion of the extract is
frozen in liquid nitrogen; a portion is assayed immediately.

Example 4. Method for assaying EPSPS activities in crude plant materials and

30   **discriminating the proportion of the total which is resistant to glyphosate.**

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EPSPS assays of plant extracts are carried out, as described above, with 0.1 mM ^{14}C-PEP and 0.75 mM shikimate-3-Pi either in the absence or the presence of 0.1 mM N-(phosphonomethyl)glycine. Under these assay conditions, the resistant forms of EPSPS of the current invention are estimated to be inhibited by < 8.5% whilst the sensitive w/t form is

5   essentially fully inhibited (> 98%). Thus, the level of activity observed in the presence of glyphosate (A) is taken to represent ~ 92% of the level of resistant enzyme derived from expression of the transgene whilst the level of susceptible w/t EPSPS is taken to be the total level of EPSPS activity observed in the absence of glyphosate minus the value of A x ~ 1.08. Because the Vmax of the mutant enzymes of the current invention are estimated to be, for

10   example, only about a third of the Vmax of the w/t enzyme (and because the Km values for PEP of both w/t and mutant forms are estimated to be about 20 μM or less), the level of expression of the mutant enzyme polypeptide relative to the level of expression of the endogenous w/t EPSPS is taken, to be about three fold higher than the ratio calculated on the basis of the ratio of their relative observed activities. The total level of EPSPS polypeptide

15   expression (mutant + w/t) is also estimated by Western blotting.

Example 5.   Cloning and expression of w/t cDNA encoding mature Brassica napus EPSPS in E.coli.

Brassica napus EPSPS cDNA is amplified using RT-PCR from RNA isolated from glass-house grown Brassica napus plants using Superscript RT from BRL according to the

20   recommendation supplied by the manufacturer. PCR is performed using Pfu turbo polymerase from Stratagene according to the methods supplied by the manufacturer. Suitably designed oligonucleotide primers based on the known nucleotide sequence (EMBL accession X51475) are used in the amplification reaction and the reverse transcription steps. The PCR product is cloned into pCRBlunt II using Invitrogens Zero Blunt TOPO kit. The

25   sequence of the insert is confirmed by sequencing and it is verified that the predicted open reading frame corresponds to that of the predicted mature chloroplastic Brassica napus EPSPS protein with the exception of the presence of an initiating Met. The cloned and verified EPSPS sequence is excised using suitable restriction enzymes as known in the art and the purified fragment is cloned into pET24a (Novagen) digested similarly. The

30   recombinant clones are introduced into BL21 (DE3) a codon-optimised RP strain of E.coli supplied by Stratagene. The EPSPS protein is expressed in this strain following addition of 1

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mM IPTG to the fermenter medium (LB supplemented with 100ug/ml Kanamycin). The
recombinant protein of the correct predicted mass is identified (i) on the basis of Coomassie
staining of SDS gels of cell extracts and side by side comparison with Coomassie-stained
gels of extracts of similar *E.coli* cells transformed with an empty pET24a vector and ii) by
5   western analysis using a polyclonal antibody raised to previously-purified plant EPSPS
protein.

**Example 6. Cloning and expression in E.coli of glyphosate-resistant mutant Brassica
napus EPSPS**

The *Brassica napus* EPSPS cDNA in pCRBlunt is used as a template for two further
10   PCR using primer pairs designed to introduce specific changes such that the resulting .
reaction product encodes a modified EPSPS protein wherein a first position is mutated so
that the residue at this position is Ile rather than Thr and a second position is mutated so that
the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS
sequences which comprise the conserved region GNAGTAMRPLTAAV in the wild type
15   enzyme such that the modified sequence reads GNAGIAMRSLTAAV. The sequence of
Brassica napus EPSPS gene is known and methods for designing primers to effect this
mutagenesis are well-known in the art.

The resultant PCR product is gel purified and cloned into pCRBlunt II using Invitrogens
Zero Blunt TOPO kit. It is confirmed that the DNA sequence of the insert and its predicted
20   open reading frame correspond to that of the predicted mature chloroplastic Brassica napus
EPSPS protein (with the exception of the presence of an initiating Met) and also that the
desired changes (the specific mutation of T to I and P to S at specific positions in the EPSPS
sequence) are encoded . The thus cloned and verified B.napus EPSPS sequence is excised
using suitable restriction enzymes and the purified fragment cloned into pET24a (Novagen)
25   digested similarly. The recombinant clones are introduced into BL21 (DE3), a codon
optimised RP strain of *E.coli* supplied by Stratagene. The EPSPS protein is expressed in this
strain following addition of 1 mM IPTG to the fermenter medium (LB supplemented with
100ug/ml Kanamycin). The recombinant protein of the correct predicted mass is identified i)
on the basis of Coomassie staining of SDS gels of cell extracts and side by side comparison
30   with Coomassie-stained gels of extracts of similar *E.coli* cells transformed with an empty
pET24a vector and ii) by Western analysis using a polyclonal antibody raised to previously-

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purified plant EPSPS protein. This mutant form of *Brassica napus* EPSPS is purified and

characterised as described herein.

Example 7. Purification and characterisation of mutant Brassica napus EPSPS

The mature mutant *Brassica napus* EPSPS protein is purified at ~ 4 C as follows. 25

g wet weight of cells are washed in 50 ml of 0.1M Hepes/ KOH buffer at pH 7.5 containing 5

mM DTT, 2 mM EDTA and 20% v/v glycerol. Following low-speed centrifugation, the cell

pellet is resuspended in 50 ml of the same buffer but also containing 2 mM of 'Pefabloc' a

serine protease inhibitor. Cells are evenly suspended using a glass homogenizer and then

disrupted at 10000 psi using a Constant Systems (Budbrooke Rd, Warwick, U.K.) Basic Z

cell disrupter. The crude extract is centrifuged at ~ 30,000 g for 1 h and the pellet discarded.

Protamine sulphate (salmine) is added to a final concentration of 0.2% , mixed and the

solution left to stand for 30 min. Precipitated material is removed by centrifugation for 30

min at ~ 30,000 g. Aristar grade ammonium sulfate is added to a final concentration of 40%

of saturation, stirred for 30 min and then centrifuged at ~ 27,000 g for 30 min. The pellet is

resuspended in ~ 10 ml of the same buffer as used for cell disruption, further ammonium

sulfate is added to bring the solution to ~ 70% of saturation, the solution is stirred for 30 min

and centrifuged again to yield a pellet which is resuspended in ~ 15 ml of S200 buffer (10

mM Hepes/ KOH (pH 7.8) containing 1 mM DTT, 1 mM EDTA and 20% v/v glycerol).

This is filtered (0.45 micron) loaded and chromatographed down a K26/ 60 column

containing Superdex 200 equilibrated with S200 buffer. EPSPS-containing fractions

detected on the basis of EPSPS enzyme activity are combined and loaded onto an xk16

column containing 20 ml of HP Q-Sepharose equilibrated with S200 buffer. The column is

washed with S200 buffer and then EPSPS eluted within a linear gradient developed from

0.0M to 0.2M KCl in the same buffer. EPSPS elutes within a single peak corresponding to a

salt concentration at or below approximately 0.1 M. EPSPS-containing fractions detected on

the basis of EPSPS enzyme activity are combined and loaded onto a HiLoad xk26/60 column

of Superdex 75 equilibrated with Superdex 75 buffer (25 mM Hepes/ KOH (pH 7.5)

containing 2 mM DTT, 1 mM EDTA and 10% v/v glycerol. EPSPS-containing fractions

identified on the basis of enzyme activity are combined and loaded onto a 1ml column of

MonoQ equilibrated with the same, Superdex 75 buffer. The column is washed with starting

buffer and EPSPS eluted as a single peak over the course of a 15 ml linear gradient

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developed between 0.0 and 0.2M KCl. EPSPS is obtained near (>90%) pure at this stage in

the purification.. Optionally, EPSPS is further purified by exchange into Superdex 75 buffer

containing 1.0 M (Aristar) ammonium sulphate and loading onto a 10 ml column of phenyl

sepharose equilibrated in the same buffer. EPSPS is eluted as a single peak early during the

5   course of a linear gradient of declining ammonium sulphate developed between 1.0 and 0.0

M ammonium sulphate.

The purified mutant form of *Brassica napus* EPSPS, obtained by these or by similar

methods and assayed as described above in the presence of 2 mM shikimate-3-Pi, has an

apparent Vmax of ~ 5 μmol/ min/ mg and a Km for PEP of ~ 25 μM. At 40 μM PEP, the

10   IC50 value for the potassium salt of glyphosate is ~ 0.6 mM. The estimated Ki value for

potassium glyphosate of the mutant EPSPS is ~ 0.45 mM.

Example 8. Cloning of genomic DNA encoding *Brassica napus* EPSPS

A *Brassica napus* genomic DNA fragment (SEQ ID No.1), encoding EPSPS is

known in the art (Gasser and Klee, 1990). PCR primers BnEPSPS5 (SEQ ID No.2) and

15   BnEPSPS3 (SEQ ID No.3) are used to amplify the EPSPS gene from genomic DNA isolated

from *Brassica napus* var. Westar.

Pfu polymerase is used to perform the PCR using the following conditions:

94 °C 5 min

94 °C 1min

20   55 °C 30s

72 °C 12 min

The resulting 3.8 Kb product is cloned into pCR4Blunt-Topo and sequenced using

the following primers: M13Forward, M13Reverse (Invitrogen), BnF1 (SEQ ID No.4), BnF2

(SEQ ID No.5), BnF3 (SEQ ID No.6), BnF4 (SEQ ID No.7), BnF5 (SEQ ID No.8), BnF6

25   (SEQ ID No.9), BnF7 (SEQ ID No.10), BnF8 (SEQ ID No.11), BnF9 (SEQ ID No.12),

BnF10 (SEQ ID No.13), BnF11 (SEQ ID No.14) and BnF12 (SEQ ID No.15).

Example 9. Introduction of mutations into genomic DNA encoding BnEPSPS.

The desired Thr-Ile and Pro-Ser mutations are introduced into the B. napus EPSPS

gene by PCR using oligonucleotide primers containing codon changes so as to introduce the

30   desired amino acid changes. Primers BnXho (SEQ ID No.16) and BnMUTBot (SEQ ID

No.17) are used to amplify a desired region of EPSPS from Westar genomic DNA. Likewise,

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primers BnMutTop (SEQ ID No.18) and BnNde (SEQ ID No.19) are used to amplify an

additional region from Westar genomic DNA. 1 ul of each of the PCR products is mixed and

overlapping complementary sequences allowed to anneal. The products are then joined by

PCR using primers BnXho and BnNde. The resulting PCR product is cloned and sequenced

5   to check for the inclusion of the double mutation. The Xho1/Nde1 fragment in the BnEPSPS

clone is excised and replace with the cloned Xho1/Nde1 fragment containing the double

mutant.

**Example 10. Construct for expression of mutant B.napus EPSPS in plants having the
Rubisco leader sequence upstream of the gene encoding mutant Brassica napus EPSPS.**

10   The tobacco rubisco leader is introduced into the construct by PCR. Primers

BnRBSEP (SEQ ID No.20) and Brasr12 (SEQ ID No.21) are used to amplify a product from

50 ng wildtype *B. napus* genomic DNA as template using Pfu-Turbo polymerase. The

resulting PCR product is cloned into pCR4 blunt TOPO and clones are identified by

restrictions digest that are orientated such as it may be excised as Not1 (from vector) and

15   Xho1. The rubisco leader is then introduced into the mutated BnEPSPS clone as Not1/Xho1.

**Example 11. Identification of the *Brassica napus* EPSPS promoter (useful for
expression of EPSPS in plants) by genome walking**

Increased lengths of upstream or downstream sequence of any plant gene may be

obtained using the genome walking kit as supplied and directed by Clontech.

20   The primers depicted in SEQ ID Nos 22 and 23 (Bras rl1 and Bnapus start rev) are used to

obtain upstream sequence of the *Brasicca napus* EPSPS gene using *Brasicca napus* genomic

DNA according to the methods described in the protocol accompanying the genome walker

kit.

The Bras rl1 (SEQ ID No. 22) primer is used in conjunction with the AP1 primer

25   supplied in the genome walker kit and the Bnapus start rev primer is used with the AP2

primer supplied with the genome walker kit. Products are cloned into pCR- TOPO using a

TOPO TA Cloning kit from Invitrogen according to the manufacturers instructions. PCR

using M13 forward and reverse primers is performed using Ready to Go Beads from

Pharmacia to ascertain which clones contain inserts of the correct size. A number of these

30   clones are used to make DNA preps and these DNAs are then used to determine the

nucleotide sequence of the inserts.

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A second set of primers (SEQ ID Nos.24 and 25 respectively) is used for a further

walk as shown below:

Genome walker 1 (SEQ ID No.24) with the AP1 primer

Genome walker 2 (SEQ ID No.25) with the AP2 primer

5   Additional genomic digests are performed in addition to those recommended by

Clontech's protocols. These digests include Alu I, Hae III, Hinc II and Rsa I. These digested

DNAs are then treated identically to the other digests performed as described by the

protocols accompanying the Genome walking kit. This is done to increase the chance of

getting a correct and long PCR product. Again the PCR products are cloned into pCR-

10   TOPO using a TOPO TA Cloning kit from Invitrogen according to the manufactures

instructions and sequence determination is performed on random clones obtained from each

cloned product that contain inserts of the correct size. The sequence of the inserts are

determined. Approximately 1kb of sequence upstream form the translational start site of the

Brasicca napus EPSPS is obtained that is contiguous with the published sequence.

15   **Example 12. Constructs for expression of mutant Brassica napus EPSPS in plants**

The 35S enhancer is amplified from vector pMJB1 (Figure 1) by PCR using primers

CaMV343 and CaMV46C (SEQ ID Nos.26 and 27 respectively)

The PCR product is cloned into vector pCR4Blunt-TOPO and sequenced. The

desired promoter, obtained as described in the previous example is amplified from *B. napus*

20   Westar genomic DNA using a 5' primer containing a Sph1 site and BnGUSXma (SEQ ID

No.28)

The PCR product is digested directly with Sph1 and Xma1 and ligated into the

pCR4Blunt-TOPO vector containing the 35S enhancer as Sph1/Xma1 (partial digest with

Sph 1). The CaMV35S enhancer fused to the BnEPSPS promoter is then excised from pCR4-

25   Blunt using Not1 (from vector) and Xma 1 and cloned into the PCR4Blunt vector containing

the tobacco rubisco leader and mutated B. napus EPSPS gene. The FMV enhancer (SEQ ID

No.29) or the CaMV35S enhancer (SEQ ID No.30) is synthesised chemically and subcloned

into vector pCR4 Blunt TOPO. The FMV enhancer is excised from PCR4-Blunt as

Not1/Sac1 and ligated into the CaMV35S enhancer/B. napus promoter / rubisco leader / B.

30   napus EPSPS as *Not1/Sac1*. The final construct is shown in Figure 2. The whole expression

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cassette may be excised using *EcoR1 and* ligated into a suitable vector for plant
transformation.

Example 13. Plant Transformation and Regeneration.

The expression cassette is excised from the pFCBnEPSPS vector using *EcoR1* and

5   ligated into, for example, a binary vector pBin19 at the unique *EcoR1* restriction site. The
binary vector, containing the desired expression cassette transformed into *Agrobacterium
tumefaciens* strain LBA 4404 using the freeze thaw method of transformation provided by
Holsters *et al.,* 1978. Tobacco transformation and whole plant regeneration is performed
using Nicotiana tabacum var. Samsun according to protocols in Draper *et al* (Plant Genetic

10   Transformation, Blackwell Sci. Pub. 1989). Transformation events are selected on MS-media
containing kanamycin. Alternatively pFCBnEPSPS, or other constructs capable of
delivering glyphosate resistance in plants by the expression of a glyphosate resistant EPSPS
gene from soybean may be introduced into plants directly without using *Agrobacterium*
mediated techniques as described for Soybean.

15   Constructs are transformed into regenerable embryogenic soyabean tissues using
either biolistic type approaches (e.g Santarem ER, Finer, J.J (1999) 'Transformation of
soyabean (*Glycine max* (L.) Merrill) using proliferative embryogenic tissue maintained on a
semi-solid medium' In vitro Cellular and Developmental Biology-Plant 35, 451-455; USP-
5,503,998, USP 5830728)or via infection with Agrobacterium (e.g. USP-5,024,944, USP-

20   5,959,179). Regenerable embryogenic soyabean tissues are derived, for example, from the
cotyledons of immature embryos or other suitable tissues.

Proliferative embryogenic tissue can, for example, be maintained on a semi-solid
medium. Such tissue, is, for example obtained in the following way. Immature zygotic
embryos which are 3- 4 mm long are isolated from pods of, for example, *Glycine max* (L.)

25   Merrill, 2-3 weeks after flower formation.   Pods can be checked for the presence of embryos
of the correct length and maturity by 'backlighting'. Pods are then sterilized. Immature
embryos are removed and the axis removed from each. Immature embryos are then plated on
'D40-Lite' semi-solid (0.2% gelrite) MS salts medium at pH 7.0 containing B5 vitamins, 3%
sucrose and 40 mg/l 2,4-D for 3-4 weeks. For proliferation of embryos the material is then

30   transferred to 'D20' MS salts medium at pH 5.7 containing B5 vitamins, 3% sucrose, 20

mg/l 2,4-D and 0.2% Gelrite. Material with bright green globular proliferative embryos is selected and subcultured every 2-3 weeks.

For bombardment, 20-25 clumps/ plate of tissue are selected (subcultured 4-5 days prior to bombardment) and arranged in the centre of the dish containing D20 medium. The
5   tissue is dried for 15 min by uncovering for 15 minutes under a sterile hood. Gold particles coated in DNA construct (coated, for example, using methods described in the references above) are twice bombarded into the tissue on D20 medium using any one of a large number of commercially available guns. By way of further example a PDS1000 particle gun is used. Particles may be prepared and coated with DNA in a similar manner to that described by
10   Klein *et al* 1987, Nature, 327, 70-73. Alternatively, for example, 60 mg of gold or tungsten particles (~ 1.0 μm) in a microcentrifuge tube are washed repeatedly in HPLC-grade ethanol and then, repeatedly, in sterile water. The particles are resuspended in 1 ml of sterile water and dispensed into 50 μl aliquots in microcentrifuge tubes. Gold particles are stored at 4 C, tungsten particles at - 20 C. 3 mg of DNA are added to each aliquot of (defrosted) particles
15   and the tubes are vortexed at top speed. Whilst maintaining near continuous vortexing, 50 μl of 2.5M $CaCl_2$ and 20 μl of 0.1M spermidine is added. After 10 minutes of further vortexing, samples are centrifuged for 5 seconds in an eppendorf microcentrifuge, the supernatant is drawn off and the particles washed in successive additions of HPLC-grade ethanol. The particles are thoroughly resuspended in 60 μl of ethanol and then dispensed in
20   10 μl aliquots onto the surface of each macrocarrier to be used in the PDS1000 particle gun. Components of the PDS1000 particle gun are surface sterilised by immersion in 70% ethanol and air-drying. Target plates prepared, as described above, with tissue arranged into an ~ 2.5 cm disc are placed 6 cm from the stopping screen. Suitably chosen rupture discs are then used for bombardment.

25   One week after bombardment, all tissue clumps are transferred onto D20 medium, buffered to pH 5.7, containing a suitable selective concentration of glyphosate between 0.05 and 5 mM. After an additional 3-4 weeks all tissue is transferred to fresh D20 medium containing an increased concentration of glyphosate within this concentration range. After a further 3-4 weeks, living tissue is selected and subcultured on every 3-4 weeks in similar
30   D20 medium containing glyphosate. Alternatively, in the case that some other selectable marker than glyphosate is also present then selections may be made as appropriate (e.g using

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increasing concentrations of hygromycin). Growing sections are thus maintained and, given enough tissue, may be analysed by PCR to confirm that they are transgenic for the desired DNA.

In order to develop and mature embryos, tissue clumps are placed onto M6 medium which comprises MS salts at pH 5.7 containing B5 vitamins, 6% maltose and 0.2% gelrite. 6-9 clumps are placed in a tall dish at 23°C. After 3-4 weeks, embryos elongate and can be separated and transferred to another round of incubation on M6 medium. After 4-6 weeks, embryos are cream-coloured and ready for desiccation. 9 such cream-coloured embryos are placed in a dry Petri dish, sealed with parafilm and placed onto a shelf for 2-3 days. Embryos should be somewhat flaccid and not "crispy-crunchy".

Dessicated embryos can be germinated by plating onto OMS (growth regulator-free MS medium). Following germination which normally occurs within a week plants are transferred to larger boxes and, once there is sufficient root and shoot formation, thence to soil. To prevent fungal contamination it is advisable to wash OMS from the roots with distilled water. Plants may be kept and grown under high humidity and, initially, under 24 hour lighting. Plants may be grown until about 2 feet tall under 24 hour lighting and then encouraged to flower and form pods through a shift to a 16 hour lighting regime. Seeds are collected and progeny grown on, crossed and backcrossed into order to move the transgenes into the desired plant background using the normal methods of plant breeding. Plants are routinely analysed for the presence and expression of transgenes using the normal methods of molecular biology including analysis by PCR, Southern, Western, ELISA and enzyme assay techniques.

Example 14. Methods of obtaining genes encoding stable glyphosate resistant EPSPs

In one embodiment of the current invention, glyphosate resistant EPSPS genes suitable for expression in E.coli are constructed, as for example, described herein where, in the coding sequence a first position is mutated so that the residue at this position is Ile rather than Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the following conserved region GNAGTAMRPL in the wild type enzyme such that the modified sequence reads GNAGIAMRSL.

Stable glyphosate-resistant EPSP synthases of the current invention and genes
encoding these are then obtained by generating a wide range of variants by a process of
random or partially random mutagenesis (for example using chemical or UV mutagenesis, by
using a non-proof reading DNA polymerase (e.g. in a strain such as XL1 red) or by using any

5   one of a number of 'directed evolution' approaches (e.g as reviewed by Kuchner and Arnold,
1997 in TIBTECH, 15, 523-530 or described by Stemmer (1994) in PNAS, 91, 10747-
10751) and selecting genes expressing EPSPSs which are stable and which remain
glyphosate resistant. Suitable means of selection include, for example, expression of
mutated genes (e.g from a weak constitutive promoter) in an Aro A- strain of E.coli grown at

10   elevated temperatures (e.g 37-42 C) and selecting those transformants most capable of
growth in minimal medium (optionally also containing glyphosate). Alternatively, a non-aro
A host strain might be used (preferably e.g. rec A) provided that the medium contains a
selective concentration of glyphosate. The skilled man will recognise that many methods of
mutagenesis and selection could be used within the ambit of the current invention.

15   **Example 15. Stable glyphosate-resistant EPSPSs and genes encoding them**

The stable double mutant form of Brassica napus EPSPS is one example of the
current invention. The skilled man will recognise that other examples of such EPSPSs of the
current invention and genes encoding them can be cloned, isolated and characterised using
methods the same or similar to those described above.

20   In EPSPSs and the EPSPS gene sequences of the current invention, the coding
sequence at a first position is mutated so that the residue at this position is Ile rather than Thr
and a second position is mutated so that the residue at this position is Ser rather than Pro, the
mutations being introduced into EPSPS sequences which comprise the following conserved
region GNAGTAMRPL in the wild type enzyme such that the modified sequence reads

25   GNAGIAMRSL.

EPSPS protein sequences of the current invention are further characterised in that
they DO NOT comprise a region having the sequence K(A/V)AKRAVVVGCGGKFPVE

EPSPS protein sequences of the current invention are further characterised in that
they comprise at least one of the amino acid sequence motifs listed below in (i)→ (viii).

30   (i) ALLMZAPLA   where Z is NOT S or T

(ii) EIEIZDKL   where Z is NOT V

    (iii) FG(V/I)(K/S)ZEH   where Z is NOT V

    (iv) AL(K/R)ZLGL   where Z is NOT R

    (v) GLXVEZ_1DXZ_2XXXA(I/V)V   where Z_1 is NOT T and/or Z_2 is NOT E

    (vi) ITPPZ_1K(L/V)(K/N)Z_2 where Z_1 is NOT K and/or Z_2 is NOT T

5   (vii) TIZ(D/N)PGCT   where Z is NOT N or L

    (viii) (D/N)YFXVLXZXX(K/R)H   where Z is NOT R

wherein X is any amino acid and Z, Z1, and Z2 are any amino acid other than those indicated.

    Furthermore it is preferred, for EPSPS genes and proteins of the current invention,

10   that the protein sequence comprise at least one of the sequence motifs i), ii) iii), v) and vi), preferably, at least two of these.

    Furthermore, in amino acid sequence motif i) Z is preferably A, in sequence motif ii) Z is preferably I, in sequence motif iii) Z is preferably A, in sequence motif iv) Z is preferably K, T or A, in sequence v) Z_1 is preferably R or A and less preferably D or E whilst

15   Z_2 is preferably V or A and less preferably T, in sequence motif vi) Z_1 is preferably E or A whilst Z_2 is preferably P, I or V, in sequence motif vii) Z is preferably R or K and in sequence motif viii) Z is preferably T or S and less preferably is Q.

    Furthermore, some of the EPSPS protein sequences of the current invention are characterised in that they comprise at least one of the following sequences:

20   (I/V)VEGCGG(I/L/Q)FP(V/A/T)(S/G) or

    (I/V)VVGCGG(I/L/Q)FP(V/A/T)E or

    (I/V)VVGCGG(I/L/Q)FP(V/A/T)(S/G) or

    (I/V)VV GCGGKFP(V/A/T)(S/G)or

    (I/V)VEGCGGKFP(V/A/T)E or

25   (I/V)VEGCGG(I/L/Q)FP(V/A/T)E or

    (I/V)VEGCGGKFP(V/A/T)(S/G)

    Furthermore EPSPS sequences of the current invention are preferably derived from dicotyledenous plants

Example 16.   Stable glyphosate-resistant EPSPSs derived from soyabean

30   Preferred examples of the current invention are the EPSPS protein sequences and the DNA sequences which encode these which are derived from soyabean (see for example

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EPSPS gene SEQ ID Nos 33 and 34) and which are then optionally modified according to Example 15 to yield a stable glyphosate resistant EPSP synthase.

Particularly preferred examples are EPSP synthase genes derived from soyabean in which:-

5   (a)   the coding sequence at a first position is mutated so that the residue at this position is Ile rather than Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the following conserved region GNAGTAMRPL in the wild type enzyme such that the modified sequence reads GNAGIAMRSL,

10 And

      (b)   the DNA coding sequence may be further modified in accord with Example 15 so that the encoded protein comprises at least one and preferably two or more of the amino acid sequence motifs ii), iii), v) and viii) .

Example 17 Cloning of a soybean partial length cDNA encoding EPSPS.

15   Soybean RNA is isolated using TriZol™ reagent as described by Gibco BRL and first-strand cDNA synthesised using Superscript II (Gibco BRL) using protocols provided by the manufacturer with the degenerate primer EPSPS 10 reverse (SEQ ID No.35). PCR is performed using ready to go PCR beads supplied by AmershamPharmacia™ according to the protocol supplied by the manufacturer using the reverse transcribed soybean first-strand

20 cDNA product as a template and the primers EPSPS 4 forward (SEQ ID No.34) and EPSPS 10 reverse (SEQ ID No.33).

The following PCR conditions are used:-

1. 94°C 3 minutes for 1 cycle

2. 94°C 45 seconds

25   50°C 30 seconds

      72°C 1 minute for 25 cycles

3. 72°C 10 minutes for 1 cycle.

The product obtained of approximately 1 kb is cloned into pCR2.1 using a TOPO TA cloning Kit (Invitrogen) according to the manufacturer's recommendations. Colonies are

30 picked at random and are selected for further work via plasmid preparation with the QIAprep Mini-Prep Kit (Qiagen), followed by digestion with *Eco* RI to show the size of the insert in

these clones. Clones containing the expected 1kb insert are progressed. The polynucleotide

sequence of selected clones is determined using an ABI 377 automated sequencer. Database

searching is performed using the Blast algorithm (Altschul *et al.*, 1990) and indicates that

the majority of sequenced clones contain partial length cDNAs exhibiting high homology

5   toward known plant EPSPS sequences.

Example 18 Isolation of a genomic polynucleotide sequence encoding soybean EPSPS.

A soybean genomic library constructed in Lambda Fix II is purchased from

Stratagene and 1,000,000 clones are plated out according to the suppliers protocols. Hybond

N+ filters are used to make lifts from the library plates and these were processed according

10   to the protocols supplied by AmershamPharmacia and Molecular Cloning - a laboratory

manual (Sambrook et al., 1989). UV cross linking of the DNA to the membrane is carried

out using a UV Stratalinker (Stratagene). The soybean EPSPS cDNA probe is prepared by

digesting the clone containing the soybean EPSPS partial length cDNA sequence with *Eco

RI* and purifying the 1 kb product by agarose gel electrophoresis. The probe is labelled with

15   ^{32}P dCTP using a Ready-To-Go™ DNA labelling kit (AmershamPharmacia). The filters are

prehybridised and hybridised in Rapid-Hyb Buffer (Amersham Pharmacia). Washes are

performed using 0.1 x SSPE, 0.5% SDS at 65 °C. The filters are then wrapped in Saran wrap

and exposed to film in a cassette containing an intensifying screen as described in Molecular

Cloning - a laboratory manual (Sambrook et al., 1989).

20   Hybridising plaques are picked into SM buffer, replated at lower density and

rescreened through 2 further rounds of purification. Phage DNA is recovered from plaque

pure phage stocks using methods well known in the art (Sambrooke et al. 1989). The

recovered phage DNA is digested with *Not* 1, and the genomic DNA subcloned into

pBluescript. The resulting vector is transformed into TOP 10 *E.coli*. The resulting clones are

25   sequenced using the Genome Priming System (NEB) using protocols provided by the

manufacturer. Briefly, sufficient batches of 96 GPS events are selected and DNA prepared

using a Qiagen™ Biorobot. The DNA was sequenced with the GPS-S and GPS-N primers as

supplied by NEB using an ABI 377 automated sequencer. Sequence data are analysed using

Seqman (DNASTAR Inc). The gene encoding EPSPS is identified by homology to known

30   EPSPS genes using the Blast algorithm. The sequence of two contigs containing sequences

showing high homology to known EPSPS are given in SEQ ID No.37 and SEQ ID No.38

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and are termed GmEPSPS1/14 and GmEPSPS12 respectively. A schematic representation of
these genes is given in Figure 3 and Figure 4.

The person skilled in the art will know that there could be other EPSPS genes present
in soybean and that these may be isolated and the nucleotide sequence determined using
5   similar methodologies as described in this example.

Example 19 Production of plant transformation vector harbouring the soybean EPSPS

gene.

The two enhancer combinations FMV35S90 (SEQ ID No.37 and FMV35S46 (SEQ
ID No.38) are synthesised chemically and subcloned into vector pTCV1001 (Figure 5) using
10   *Hind* III / *Pac* 1. The soybean EPSPS terminator, and a small region of the coding sequence,
are obtained by PCR using the primers GmTermBam (SEQ ID No.39) and GmTermEco
(SEQ ID No.40) using clone GmEPSPS 1/14 as template. The PCR product is cloned into
pCR4-Blunt-TOPO, sequenced, and excised using *Pst*1 and *Eco*R1 and ligated into the
pTCV1001 vector containing the FMV/CaMV35S enhancers. Finally, the Pac1:BamH1
15   DNA fragment containing the majority of the soybean EPSPS gene in either GmEPSPS 1/14
is excised and cloned into the pTCV1001 vector comprising both the enhancers and
terminator.

Example 20 Introduction of mutation into EPSPS gene.

The desired Thr to Ile and Pro to Ser mutations required to increase tolerance to
20   glyphosate are inserted into the construct in the following manner. Primer BnMUT2PST
(SEQ ID No.41), which contains the nucleotide changes that give rise to the desired amino-
acid mutation, is used in conjunction with primer BnMUTKPN (SEQ ID No.42) in PCR with
pfu-TURBO polymerase (Stratagene) with GmEPSPS 1/14 as template. The PCR product,
now containing the desired mutation, is cloned into pCR4-Blunt-TOPO and sequenced. It is
25   then excised as *Kpn* 1 *Pst* 1 and used to replace the corresponding fragment in the construct
in pTCV1001 containing WT sequence. The final vector is termed pTCVGMEPSPS (Figure
6).

Example 21   Introduction of 5' UTL.

An additional 5' UTL, such as that from the small subunit of ribulose biphosphate
30   carboxylase/oxygenase (Rubisco) or tobacco glucanase can be inserted into the construct by
PCR. In addition the context surrounding the AUG translation initiation codon is optimised

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to give the consensus Kozak sequence ACCAUGG. With regard the SSU rubisco leader, primers GmRubPac (SEQ ID No.43) and GmRubbot (SEQ ID No.44) are used to introduce the soybean rubisco 5' UTL into 5' UTL of the soybean EPSPS gene. GmRubTop (SEQ ID No.45) and GmRubKpn (SEQ ID No.46) are then used to introduce the soybean rubisco 5'

5   UTL into the first exon of the soybean EPSPS gene. The two PCR products are then joined, by PCR using primers GmRubPac and GmRubKpn and the resulting product cloned into pCR4Blunt-TOPO and sequenced. Once the authenticity of the PCR product is confirmed it is excised from the PCR4 vector as *Pac*1:*Kpn*1 and used to replace the *Pac*1/*Kpn*1 restriction fragment in vector pTCVGMEPSPS.

10   These examples outline the construction of a DNA sequence comprising two enhancers fused to a soybean EPSPS gene containing specific mutations in the coding sequence that is capable of producing a glyphosate resistant EPSPS enzyme in plants. The person skilled in the art will know that enhancers may be introduced at various distances up stream from the promoter and transcriptional start site to act to enhance the rate and/or

15   amount of transcription of the double mutant EPSPS soybean gene in plant cells. The distance may have to be determined empirically for each individual gene. This involves making a number of constructs where the enhancers are fused at different distances from the transcriptional start of the double mutant EPSPS soybean gene and introducing these constructs into plant material and assaying the material for resistance to selecting

20   concentrations of glyphosate. The person skilled in the art will also know that enhancer choices are not restricted to CaMV 35S and FMV enhancers, but may also include enhancers from any gene that is highly expressed in plants including *inter alia,* enhancers from actin genes, ubiquitin genes, mannopine, nopaline and octopine synthase genes, genes from commelina yellow virus and other plant viruses expressed in plants such as potato virus Y

25   and X coat proteins. The choice of 5' UTR may not be restricted to soybean small subunit rubisco and leader from other genes may be used.

Example 22   Plant Transformation and Regeneration.

The expression cassette is excised from the pTCVGMEPSPS vector using *Xma* 1 and ligated into a binary vector pBin19 at the unique Xma 1 restriction site. The binary vector, containing the desired expression cassette transformed into *Agrobacterium tumefaciens* strain

5   LBA 4404 using the freeze thaw method of transformation provided by Holsters *et al.*, 1978. Tobacco transformation and whole plant regeneration are performed using Nicotiana tabacum var. Samusun according to protocols in Draper *et al* (Plant Genetic Transformation, Blackwell Sci. Pub. 1989). Transformation events are selected on MS-media containing kanamycin.

10   Alternatively PTCGMEPSPS, or other constructs capable of delivering glyphosate resistance in plants by the expression of a glyphosate resistant EPSPS gene from soybean may be introduced into plants directly without using Agrobacterium mediated techniques as described for Soybean in a number of references including the following: Physiol. Plant. (1990), 79(1), 210-12; USP-5,968,830; WO-0042207; and USP-5,959,179 and as described

15   in Example 13.

Example 23   Analysis of Transgenic Plants

PCR analysis of transformants

Leaf samples were taken from transformed lines and DNA extracted according to known methods. Oligonucleotide primers are designed to specific regions within the transgene to

20   enable its detection in the plant material tested.

RNA analysis

The presence of mRNA encoding the transgene is detected within the plant using Northern Blot hybridisation. Total RNA is extracted from leaf tissue using Tri-reagent and protocols provided by the manufacturer (Sigma™). Blots are prepared using existing procedures and.

25   probed using radio-labelled soybean EPSPS cDNA.

Protein Analysis

The presence of recombinant protein in the transgenic plant is determined using Western blotting procedures with antibodies raised to recombinant soybean EPSPS using standard protocols.

30   **Enzyme assays**

Resistant EPSPS activity is detected in plants using ^{14}C radiolabelled substrates and detection of products by HPLC.

Herbicide tolerance tests

Following tissue culture, transgenic plants are transferred to 5 inch pots containing John

5   Innes potting compost no. 3. The plants are allowed to acclimatise (approx. 2 weeks) in the greenhouse and formulated glyphosate applied at various concentrations to the aerial tissue using a track spayer. Visual assessment of the transformed plants to identify those that are resistant to glyphosate is performed 21 days post application.

Example 24 Introduction of plastid targeted pyruvate orthophosphate di-kinase

10   **(PPDK) or phosphoenolpyruvate synthase (PPS) constructs.**

The cDNA encoding PPDK from maize (EMBL Accession J03901), containing its autologous transit peptide sequence, is amplified by RT-PCR from maize tissue using primers ZMPPDK1 (SEQ ID No. 47) and ZMPPDK2 (SEQ ID no. 48). The RT-PCR product

15   is cloned into the vector pCR2.1 TOPO and sequenced to check for authenticity. The maize PPDK gene is excised from the pCR2.1 vector, as a *Nco* 1/*Kpn* 1 fragment and ligated into similarly digested pMJB1 (Figure 1). The plant expression cassetee is then subcloned into the pTCVGMEPSPS cassette (Figure 6) prior to further plant transformation. Other PPDK genes, such as that encoding a cold-stable PPDK from *Flaveria brownii* (EMBL accession

20   AAQ94645) or form rice can be engineered using a similar strategy.   Likewise, the gene encoding PPS in *E. coli* (EMBL Accession X59381) is amplified by PCR using oligos ECPPS1 (SEQ Id No. 49) and ECPPS2 (SEQ ID No. 50). The PCR product is cloned into plant expression cassette containing a plant operable promoter (such as the cauliflower mosaic virus 35S promoter), a chloroplast transit signal sequence (such as that from the small

25   subunit of rubisco) and a terminator (such as NOS).

Sequence listing

Brassica napus genomic DNA encoding an EPSPS (SEQ ID No.1)

gcatgcagatcttaaaggctcttttccagtctcacctaccaaaactataagaaaatccacttgctgtctgaaatagccgacgtggataaag

30   tacttaagacgtggcacattattattgactactagaaaaaaaactcatacaccatcgtaggagttggggttggtgaagaatttgatgggtg

cctctccccccccccactcaccaaactcatgttctttgtaaagccgtcactacaacaacaaaggagacgacagttctatagaaaagctttc

aaattcaatcaatggcgcaatctagcagaatctgccatggcgtgcagaacccatgtgttatcatctccaatctctccaaatccaaccaaa

acaaatcacctttctccgtctccttgaagacgcatcagcctcgagcttcttcgtggggattgaagaagagtggaacgatgctaaacggtt

ctgtaattcgcccggttaaggtaacagcttctgtttccacgtccgagaaagcttcagagattgtgcttcaaccaatcagagaaatctcgg

gtctcattaagctacccggatccaaatctctctccaatcggatcctccttcttgccgctctatctgaggtacatatacttgcttagtgttaggc

ctttgctgtgagattttgggaactatagacaatttagtaagaatttatatataattttttaaaaaaaaatcagaagcctatatatatttaaatttttc

5   caaaattttttggaggttataggcttatgttacaccattctagtctgcatctttcggtttgagactgaagaatttattttttaaaaaattattatag

ggaactactgtagtggacaacttgttgaacagtgatgacatcaactacatgcttgatgcgttgaagaagctggggcttaacgtggaacg

tgacagtgtaaacaaccgtgcggttgttgaaggatgcggtggaatattcccagcttccttagattccaagagtgatattgagttgtacctt

gggaatgcaggaacagccatgcgtccactcaccgctgcagttacagctgcaggtggcaacgcgaggtaaggttaacgagttttttgtt

attgtcaagaaattgatcttgtgtttgatgcttttagtttggtttgtttctagttatgtacttgatggggtgcctagaatgagggaaagacctat

10   aggagatttggttgttggtcttaagcagcttggtgctgatgttgagtgtactcttggcactaactgtcctcctgttcgtgtcaatgctaatggt

ggccttcccggtggaaaggtgatcttcacatttactctatgaattgtttgcagcagtctttgttcatcacagccctttgcttcacattatttcatct

tttagtttgttgttatattacttgatggatctttaaaaaggaattgggtctggtgtgaaagtgattagcaatctttctcgattccttgcagggcc

gtgggcattactaagtgaaacattagcctattaacccccaaaattttttgaaaaaaatttagtatatggccccaaaatagtttttaaaaaatta

gaaaaactttaataaatcgtctacagtcccaaaaatcttagagccggccctgcttgtatggtttctcgattgatatattagactatgttttga

15   atttcaggtgaagctttctggatcgatcagtagtcagtacttgactgccctcctcatggcagctcctttagctcttggagacgtggagatt

gagatcattgataaactgatatctgttccatatgttgaaatgacattgaagttgatggagcgttttggtgttagtgccgagcatagtgatag

ctgggatcgtttctttgtcaagggcggtcagaaatacaagtaatgagttctttttaagttgagagttagattgaagaatgaatgactgattaa

ccaaatggcaaaactgattcaggtcgcctggtaatgcttatgtagaaggtgatgcttctagtgctagctacttcttggctggtgctgccatt

actggtgaaactgttactgtcgaaggttgtggaacaactagcctccaggtagtttatccactctgaatcatcaaatattatactccctccgtt

20   ttatgttaagtgtcattagcttttaaattttgtttcattaaaagtgtcattttacattttcaatgcatatattaaataaaattttccagttttttactaattc

attaattagcaaaatcaaacaaaaattatattaaataatgtaaaattcgtaatttgtgtgcaaataccttaaaccttatgaaacggaaacctta

tgaaacagagggagtactaattttataataaaaatttgattagttcaaagttgtgtataacatgttttgtaagaatctaagctcattctcttttatt

ttttgtgatgaatcccaaagggagatgtgaaattcgcagaggttcttgagaaaatgggatgtaaagtgtcatggacagagaacagtgtg

actgtgactggaccatcaagagatgctttggaatgaggcacttgcgtgctgttgatgtcaacatgaacaaaatgcctgatgtagccatg

25   actctagccgttgttgctctctcttgccgatggtccaaccaccatcagagatggtaaagcaaaaccctctctttgaatcagcgtgttttaaaa

gattcatggttgcttaaactctatttggtcaatgtagtggctagctggagagttaaggagacagagaggatgattgccatttgcacagag

cttagaaaggtaagtttccttttctctcatgctctctcattcgaagttaatcgttgcataacttttgcggtttttttttttgcgttcagcttggagc

tacagtggaagaaggttcagattattgtgtgataactccaccagcaaaggtgaaaccggcggagattgatacgtatgatgatcatagaa

tggcgatggcgttctcgcttgcagcttgtgctgatgttccagtcaccatcaaggatcctggctgcaccaggaagactttccctgactactt

30   ccaagtccttgaaagtatcacaaagcattaaaaagacccctttcctctgatccaaatgtgagaatctgttgctttctctttgttgccactgtaaca

tttattagaagaacaaagtgtgtgtgttaagagtgtgtttgcttgtaatgaactgagtgagatgcaatcgttgaatcagttttgggccttaat

aaagggtttaggaagctgcagcgagatgattgtttttggtcgatcatctttgaaaatgtgtttgtttgagtaattttttctagggttgagttgatt

acactaagaaacactttttgattttctattacacctatagacacttcttacatgtgacacactttgttgttggcaagcaacagattgtggacaa

ttttgcctttaatggaaaggacacagttgtggatgggtgatttgtggacgattcatgtgtgttaggtgatttgtggacggatgatgtgtag

atgagtgatgagtaatgtgtgaatatgtgatgttaatgtgtttatagtagataagtggacaaactctctgttttgattccataaaactatacaa

5   caatacgtggacatggactcatgttactaaaattataccgtaaaacgtggacacggactctgtatctccaatacaaacacttggcttcttca

gctcaattgataaattatctgcagttaaacttcaatcaagatgagaaagagatgatattgtgaatatgarscggagagagaaatcgaaga

agcgtttacctttttgtcggagagtaatgaattc
BnEPSPS5 (SEQ ID No.2)

gcatgcagatcttaaaggctcttttccagtc

10   BnEPSPS3 (SEQ'ID No.3)

gaattcattactctccgacaaaaggtaaacgc
BnF1 (SEQ ID No.4)

gcgcaatctagcagaatctg
BnF2 (SEQ ID No.5)

15   tccttcttgccgctctatct
BnF3 (SEQ ID No.6)

gcgttgaagaagctgggggct
BnF4 (SEQ ID No.7)

acctataggagatttggttg

20   BnF5 (SEQ ID No.8)

actaagtgaaacattagcct
BnF6 (SEQ ID No.9)

gttccatatgttgaaatgac
BnF7 (SEQ ID No.10)

25   tccactctgaatcatcaaat
BnF8 (SEQ ID No.11)

gtaagaatctaagctcattc
BnF9 (SEQ ID No.12)

tcatggttgcttaaactcta

30   BnF10 (SEQ ID No.13)

cagcttgtgctgatgttcca

BnF11 (SEQ ID No.14)

cgatcatctttgaaaatgtg

BnF12 (SEQ ID No.15)

ctctctgttttgattccata

5   BnXho (SEQ ID No.16)

cgcatcagcctcgagcttcttcgtg

BnMUTBot (SEQ ID No.17)

ggtgagtgaacgcatggctattcctgcat

BnMutTop (SEQ ID No.18)

10   atgcaggaatagccatgcgttcactcacc

BnNde (SEQ ID No.19)

catttcaacatatggaacagatatc

BnRBSEP (SEQ ID No.20)

cccgggatattggaagttaaaggaaaagagagaaagagaaatctttctgtctaagtgtaattaaccatggcgcaatctagcagaatctg

15   c

Brasr12 (SEQ ID No.21)

ggacgtggaaacagaagctg

Bras r11 (SEQ ID No.22)

tctcaaaccgaaagatgcag

20   Bnapus start rev (SEQ ID No.23)

ggttgaagcacaatctctgaagcttccatgg

Genome walker 1 (SEQ ID No.24)

gatcttgggtcattttaatgggccttctaac

with the AP1 primer

25   ·Genome walker 2 (SEQ ID No.25)

aaataaacccattaaccactaatgaaaaagggat

with the AP2 primer

CaMV343 (SEQ ID No.26)

gcggagctcgagactttcaacaaaggg

30   CaMV46C (SEQ ID No.27)

atcccgggaagcatgcgaaggatagtgggattgtg

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BnGUSXma (SEQ ID No.28)

cgcccgggattgaatttbaaagc

FMV enhancer (SEQ ID No.29)

tctagagcagctggcttgtggggaccagacaaaaaaggaatggtgcagaattgttaggcgcacctaccaaaagcatctttgcctttatt

gcaaagataaagcagattcctctagtacaagtggggaacaaaataacgtggaaaagagctgtcctgacagcccactcactaatgcgta

tgacgaacgcagtgacgaccacaaaagagctc

CaMV35S enhancer (SEQ ID No.30)

tgagacttttcaacaaagggtaatatccggaaacctcctcggattccattgcccagctatctgtcactttattgtgaagatagtggaaaag

gaaggtggctcctacaaatgccatcattgcgataaaggaaaggccatcgttgaagatgcctctgccgacagtggtcccaaagatgga

cccccacccacgaggagcatcgtggaaaaagaagacgttccaaccacgtcttcaaagcaagtggattgatgtgatatctccactgacg

taagggatgacgcacaatcccactatccttcgcaagacccttc

Soyabean genomic DNA encoding an EPSPS (SEQ ID No. 31)

aaaaaataccttttagtaaaataagagcgtragatcaatcagacgttttctctttgggacttctcattcttaattgaattgacaataactaaagt

gaaactaaggctaaaatcaactcgcctagtcaagctcgtccacaaaaataggtttttgaaagtttaccatgtcagtttcttactaagtaaaa

aaggatcatttgtaaggtccaacgccttaaaatgatcacccttcaaagtaaaagaatcacttgtttcacgcataagtaagaactacgtagg

tctgatttcctctccaaaggaggggtacgtaggagcaaaagccccgcttttgtcgaccttaaaaaataaaaagaaacaaaagttaaggta

acacaatttccacaattctagaaataaggttgttgtctttcgagacaaacgtaagaggtgctaataccttcctcaaacgtaaatacaactcc

cgaacttagaattttcattttgaccggtttccttcggttttcccgacgtttccacaaataaacgttggtggcgactccgcgcatctttcctcct

ttggaaagcgcacccatgagcctcgccctcgctcgcccgcgaagggcacgttgcgacactaggttaataagaagtttagtccacatat

ctatggacctcttaaggtgattatcaaagtggaagtggtgacctacatgttggccctaccagcttattcgtatatttacccccatcaaccat

gtatctcagctgaaacatgctatcaatccaaatgttatcagaggaacatgagctgcatgttcaaccagaagtagttctagtcttaatacaa

atgtcaaatggcacaagtgagattttgaatttctgatgttgtaaaaatctcaggacatgaatactattgggaagcaattattcatacttcacc

aatccaaactgacccaaaattctcaaatcacatgaaagcaaaaatgcatataacacgaagaataagaagaagaggaactaacctggg

gtttcgatgattaaagcgttgttgttgatgatgaaaacgatgattatggagagaaattgttgttgaatggtgaaattgttatagaaagagaa

cgaagatagagaaaaagatatatagattttttcaaggctcaaaccctaaaatcaccatgagagagaacaaagattgagaaacctacaac

cactatgagagagaatgagcagaacagaagcgtgagatagagaacgagagttaaggtgcgagaggacacgaagaacaaaaggtg

tgagagaaagaacaaaggagcctacggtgtgagatgagagaatttgaaattcttaccatttaggtggaatttcaattctacaattttattct

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EPSPS 4 forward (SEQ ID No.34)

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SEQ ID No. 31 with double mutation (SEQ ID No.35)

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15   ECPPS1 (SEQ ID No. 49)
gcggcatgcccaacaatggctcgtc

ECPS2 (SEQ ID No. 50)

gcggatccttatttcttcagttcagcca

20   EPSPS motif (SEQ ID No. 51)

ALLMZAPLA, wherein Z is not S or T

EPSPS motif (SEQ ID No. 52)

EIEIZDKL, wherein Z is not V

EPSPS motif (SEQ ID No. 53)

25   FG(V/I)(K/S)ZEH, wherein Z is not V

EPSPS motif (SEQ ID No. 54)

AL(K/R)ZLGL, wherein Z is not R

EPSPS motif (SEQ ID No. 55)

GLXVEZ$_1$DXZ$_2$XXXA(I/V)V, wherein Z$_1$ is not T and/or Z$_2$ is not E

30   EPSPS motif (SEQ ID No. 56)

ITPPZ$_1$K(L/V)(K/N)Z$_2$, wherein Z$_1$ is not K and/or Z$_2$ is not T

EPSPS motif (SEQ ID No. 57)

TIZ(D/N)PGCT, wherein Z is not N or L

EPSPS motif (SEQ ID No. 58)

(D/N)YFXVLXZXX(K/R)H, wherein Z is not R

EPSPS motif (SEQ ID No. 59)

(I/V)VEGCGG(I/L/Q)FP(V/A/T)(S/G)

EPSPS motif (SEQ ID No. 60)

5   (I/V)VVGCGG(I/L/Q)FP(V/A/T)E

EPSPS motif (SEQ ID No. 61)

(I/V)VVGCGG(I/L/Q)FP(V/A/T)(S/G)

EPSPS motif (SEQ ID No. 62)

(I/V)VVGCGGKFP(V/A/T)(S/G)

10   EPSPS motif (SEQ ID No. 63)

(I/V)VEGCGGKFP(V/A/T)E

EPSPS motif (SEQ ID No. 64)

(I/V)VEGCGG(I/L/Q)FP(V/A/T)E;

EPSPS motif (SEQ ID No. 65)

15   (I/V)VEGCGGKFP(V/A/T)(S/G).

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CLAIMS

1. A glyphosate resistant EPSPS enzyme wherein in comparison with the wild type EPSPS the protein sequence is modified in that a first position is mutated so that the residue at this position is Ile rather than Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the conserved region GNAGTAMRPL in the wild type enzyme such that the modified sequence reads GNAGIAMRSL, wherein the enzyme does not comprise the sequence K(A/V)AKRAVVVGCGGKFPVE, characterised in that the enzyme is stable and possesses at least one of the following sequence motifs (i) to (viii) in which X is any amino acid and Z, Z_1 and Z_2 are any amino acid other than those specified:-

   (i)   ALLMZAPLA, wherein Z is not S or T;

   (ii)   EIEIZDKL, wherein Z is not V;

   (iii) FG(V/I)(K/S)ZEH, wherein Z is not V;

   (iv)   AL(K/R)ZLGL, wherein Z is not R;

   (v)   GLXVEZ₁DXZ₂XXXA(I/V)V, wherein Z_1 is not T and/or Z_2 is not E;

   (vi)   ITPPZ₁K(L/V)(K/N)Z₂ wherein Z_1 is not K and/or Z_2 is not T;

   (vii) TIZ(D/N)PGCT, wherein Z is not N or L;

   (viii) (D/N)YFXVLXZXX(K/R)H, wherein Z is not R.

2. An enzyme according to claim 1, which comprises at least two of the motifs (i), (ii), (iii), (v) and (vi).

3. An enzyme according to either of claims 1 or 2, wherein in motif (i) Z is A; in motif (ii) Z is I; in motif (iii) Z is A; in motif (iv) Z is K, T or A; in motif (v) Z_1 is R or A or less preferably D or E and Z_2 is preferably V or A or less preferably T; in sequence motif (vi) Z_1 is E or A and Z_2 is P, I or V; in motif (vii) Z is R or K; and in motif (viii) Z is T or S or less preferably Q.

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4.   An enzyme according to claim 1, which comprises one or more sequences selected from the group consiting of:

(i)   (I/V)VEGCGG(I/L/Q)FP(V/A/T)(S/G)

(ii)   (I/V)VVGCGG(I/L/Q)FP(V/A/T)E;

(iii)   (I/V)VVGCGG(I/L/Q)FP(V/A/T)(S/G);

(iv)   (I/V)VVGCGGKFP(V/A/T)(S/G);

(v)   (I/V)VEGCGGKFP(V/A/T)E;

(vi)   (I/V)VEGCGG(I/L/Q)FP(V/A/T)E;

(vii)   (I/V)VEGCGGKFP(V/A/T)(S/G).

5.   An isolated polynucleotide comprising a region which encodes an EPSPS according to any one of claims 1 to 4.

6.   A polynucleotide according to claim 5, having a sequence depicted in SEQ ID Nos. 4 or 5.

7.   A polynucleotide encoding an EPSPS, which polynucleotide is obtainable by screening plant genomic DNA libraries with a polynucleotide constituting an intron located within SEQ ID Nos. 4 or 5.

8.   A polynucleotide according to any one of claims 5-7, comprising the following components in the 5' to 3' direction of transcription:-

(i)   At least one transcriptional enhancer being that enhancing region which is upstream from the transcriptional start of the sequence from which the enhancer is obtained and which enhancer *per se.* does not function as a promoter either in the sequence in which it is endogenously comprised or when present heterologously as part of a construct;

(ii)   The promoter from the soybean EPSPS gene;

(iii)   The soybean genomic sequence which encodes the soybean EPSPS chloroplast transit peptide;

(iv)   The genomic sequence which encodes the soybean EPSPS;

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(v)   A transcriptional terminator;

wherein the soybean EPSPS coding sequence is modified in comparison with the wild type sequence in that a first position is mutated so that the residue at this position is Ile rather than Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the conserved region GNAGTAMRPLTAAV in the wild type enzyme such that modified sequence reads GNAGIAMRSLTAAV.

9.   A polynucleotide according to claim 1, comprising the following components in the 5' to 3' direction of transcription:-

(i)   At least one transcriptional enhancer being that enhancing region which is upstream from the transcriptional start of the sequence from which the enhancer is obtained and which enhancer *per se* does not function as a promoter either in the sequence in which it is endogenously comprised or when present heterologously as part of a construct;

(ii)   The promoter from the *Brasicca napus* EPSPS gene;

(iii)   The *Brasicca napus* genomic sequence which encodes the *Brasicca napus* EPSPS chloroplast transit peptide;

(iv)   The genomic sequence which encodes the *Brasicca napus* EPSPS;

(v)   A transcriptional terminator;

wherein the *Brasicca napus* EPSPS coding sequence is modified in comparison with the wild type sequence, in that a first position is mutated so that the residue at this position is Ile rather than Thr and a second position is mutated so that the residue at this position is Ser rather than Pro, the mutations being introduced into EPSPS sequences which comprise the conserved region GNAGTAMRPLTAAV in the wild type enzyme such that modified sequence reads GNAGIAMRSLTAAV.

10.   A polynucleotide according to any one of claims 5-9, further comprising a sequence which encodes a chloroplast transit peptide/phosphoenolpyruvate synthase (CTP/PPS) or a chloroplast transit peptide/pyruvate orthophosphate di-kinase

(CTP/PPDK), which sequence is under expression control of a plant operable promoter.

11.   A polynucleotide according to the preceding claim, wherein the CTP and PPDK encoding sequences are non-heterologous with respect to each other.

12.   A polynucleotide according to any one of claims 5-11, wherein the said enhancing region constitutes a sequence, the 3' end of which is at least 40 nucleotides upstream of the closest transcriptional start of the sequence from which the enhancer is obtained.

13.   A polynucleotide according to claim 12, wherein the enhancing region constitutes a region the 3' end of which is at least 60 nucleotides upstream of the said closest start.

14.   A polynucleotide according to claim 12, wherein the said enhancing region constitutes a sequence the 3' end of which is at least 10 nucleotides upstream from the first nucleotide of the TATA consensus of the sequence from which the enhancer is obtained.

15.   A polynucleotide according to any one of claims 5-14, comprising first and second transcriptional enhancers which are tandemly present in the polynucleotide.

16.   A polynucleotide according to any one of claims 5-15, wherein the 3' end of the enhancer, or first enhancer, is between about 100 to about 1000 nucleotides upstream of the codon corresponding to the translational start of the EPSPS transit peptide, or the first nucleotide of an intron in the 5' untranslated region.

17.   A polynucleotide according to any one of claims 5-16, wherein the 3' end of the enhancer, or first enhancer, is between about 150 to about 1000 nucleotides upstream of the codon corresponding to the translational start of the EPSPS transit peptide, or the first nucleotide of an intron in the 5' untranslated region.

18.   A polynucleotide according to any one of claims 5-17, wherein the 3' end of the

         enhancer, or first enhancer, is between about 300 to about 950 nucleotides upstream

         of the codon corresponding to the translational start of the EPSPS transit peptide, or

5   the first nucleotide of an intron in the 5' untranslated region.

19.   A polynucleotide according to any one of claims 5-18, wherein the 3' end of the

         enhancer, or first enhancer, is between about 770 and about 790 nucleotides upstream

         of the codon corresponding to the translational start of the EPSPS transit peptide, or

10   the first nucleotide of an intron in the 5' untranslated region.

20.   A polynucleotide according to any one of claims 5-19, wherein the 3' end of the

         enhancer, or first enhancer, is between about 300 and about 380 nucleotides upstream

         of the codon corresponding to the translational start of the EPSPS transit peptide, or

15   the first nucleotide of an intron in the 5' untranslated region.

21.   A polynucleotide according to any one of claims 5-18 and 20, wherein the 3' end of

         the enhancer, or first enhancer, is between about 320 and about 350 nucleotides

         upstream of the codon corresponding to the translational start of the EPSPS transit

20   peptide, or the first nucleotide of an intron in the 5' untranslated region.

22.   A polynucleotide according to any one of claims 5-21, wherein the region upstream

         of the promoter from the EPSPS gene comprises at least one enhancer derived from a

         sequence which is upstream from the transcriptional start of either the CaMV35S or

25   FMV35S promoters.

23.   A polynucleotide according to any one of claims 5 -21, wherein the region upstream

         of the promoter from the EPSPS coding sequence comprises at least one enhancer

         derived from a sequence which is upstream from the transcriptional start of a

30   promoter selected from the group consisting of those of the actin, rolDFd, S-adenosyl

homocysteinase, histone, tubulin, polyubiquitin and plastocyasnin genes and the CaMV35S and FMV35S genes.

24. A polynucleotide according to claim 23, comprising in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of an actin gene.

25. A polynucleotide according to claim 23, comprising in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of the rolDfd gene.

26. A polynucleotide according to claim 23, comprising in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of a histone gene.

27. A polynucleotide according to claim 23, comprising in the 5' to 3' direction a first enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of either the CaMV35S or FMV35S promoters, and a second enhancer comprising a transcriptional enhancing region derived from a sequence which is upstream from the transcriptional start of a tubulin gene.

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28.   A polynucleotide according to claim 23, comprising in the 5' to 3' direction a first
        enhancer comprising a transcriptional enhancing region derived from a sequence
        which is upstream from the transcriptional start of the FMV35S promoter and a
        second enhancer comprising a transcriptional enhancing region derived from a
5   sequence which is upstream from the transcriptional start of the CaMV35S promoter.

29.   A polynucleotide according to any one of claims 5-28, wherein the nucleotides 5' of
        the codon which constitutes the translational start of the soybean EPSPS chloroplast
        transit peptide are Kozack preferred.

10

30.   A polynucleotide according to any one of claims 8 to 29, wherein 5' of the genomic
        sequence which encodes the EPSPS CTP there is located a 5' untranslated leader
        sequence derived from a relatively highly expressed gene

15 31.   A polynucleotide according to the preceding claim, wherein the said highly expressed
        gene is selected from the group consisting of those which encode glucanase, chalcone
        synthase and Rubisco.

32.   A polynucleotide according to any one of claims 8-31, which comprises a virally
20   derived translational enhancer or non-viral translational enhancer located within the
        non translated region 5' of the genomic sequence which encodes the EPSPS
        chloroplast transit peptide.

33.   A polynucleotide according to any one of claims 5-32, further comprising regions
25   encoding proteins capable of conferring upon plant material containing it at least one
        of the following agronomically desirable traits: resistance to insects, fungi, viruses,
        bacteria, nematodes, stress, dessication, and herbicides.

34.   A polynucleotide according to claim 33, wherein the herbicide is other than
30   glyphosate.

35.   A polynucleotide according to the preceding claim wherein the herbicide is a
protoporphyrinogen oxidase (PPGO) inhibitor, in particular butefenicil, and the
corresponding resistance protein is a PPGO or inhibitor resistant variant thereof.

5   36.   A polynucleotide according to either of claims 33 to 35, wherein the insect resistance
conferring regions encode crystal toxins derived from Bt, including secreted Bt
toxins; protease inhibitors, lectins, Xenhorabdus/Photorhabdus toxins; the fungus
resistance conferring regions are selected from the group consisting of those encoding
known AFPs, defensins, chitinases, glucanases, Avr-Cf9; the bacterial resistance
10   conferring regions are selected from the group consisting of those encoding cecropins
and techyplesin and analogues thereof; the virus resistance regions are selected from
the group consisting of genes encoding virus coat proteins, movement proteins, viral
replicases, and antisense and ribozyme sequences which are known to provide for
virus resistance; the stress, salt, and drought resistance conferring regions are selected
15   from those that encode Glutathione-S-transferase and peroxidase, the sequence which
constitutes the known CBF1 regulatory sequence and genes which are known to
provide for accumulation of trehalose.

37.   A polynucleotide according to claim 36, wherein the insect resistance conferring
20   regions are selected from the group consisting of those that encode cryIAc, cryIAb,
cry3A, cry1BC, Vip1A, Vip1B, Vip3A, Vip3B, cystein protease inhibitor, and
snowdrop lectin.

38.   A polynucleotide according to any preceding claim, which is modified in that mRNA
25   instability motifs and/or unwanted splice regions are removed, or crop preferred
codons are used so that expression of the thus modified polynucleotide in a plant
yields substantially similar protein having a substantially similar activity/function to
that obtained by expression of the protein encoding regions of the unmodified
polynucleotide in the organism in which they are endogenous.

30

39.   A polynucleotide according to the preceding claim, wherein the degree of identity
       between the modified polynucleotide and a polynucleotide endogenously contained
       within the said plant and encoding substantially the same protein is such as to prevent
       co-suppression between the modified and endogenous sequences.

40.   A polynucleotide according to the preceding claim, wherein the said degree is less
       than about 70%.

41.   A vector comprising the polynucleotide of any preceding claim.

42.   Plant material which has been transformed with the polynucleotide of any one of
       claims 5-40, or the vector of claim 41.

43.   Plant material which has been transformed with the polynucleotide of any one of
       claims 5-40 or the vector of claim 41, and which has been, or is, further transformed
       with a polynucleotide comprising regions encoding proteins capable of conferring
       upon plant material containing it at least one of the following agronomically desirable
       traits: resistance to insects, fungi, viruses, bacteria, nematodes, stress, desiccation,
       and herbicides.

44.   Morphologically normal, fertile whole plants which have been regenerated from the
       material according to either of claims 42 or 43, their progeny seeds and parts.

45.   Morphologically normal fertile whole plants which result from the crossing of plnts
       which have been regenerated from material which has been transformed with the
       polynucleotide of any one of claims 5-40 and plants which result from regeneration
       of material transformed with a polyncleotide which comprises a sequence which
       encodes a protein capable of providing for elevated levels of phosphoenolpyruvate in
       the chloroplast.

46.   Plants according to the preceding claim, wherein the said capable protein is a fusion
        of a CTP and PPS or a fusion of a CTP and a PPDK.

47.   Morphologically normal fertile whole plants which comprise the polynucleotide of
        any one of claims 5-40 and which result from the crossing of plants which have been
        regenerated from material transformed with the polynucleotide of any one of claims 5
        - 40 or the vector of claim 41, and plants which have been transformed with a
        polynucleotide comprising regions encoding a CTP/PPS or CTPPPDK, and/or
        regions encoding proteins capable of conferring upon plant material containing it at
        least one of the following agronomically desirable traits: resistance to insects, fungi,
        viruses, bacteria, nematodes, stress, desiccation, and herbicides, the progeny of the
        resultant plants, their seeds and parts.

48.   Plants according to any one of claims 44 to 47, selected from the group consisting of
        field crops, fruits and vegetables such as canola, sunflower, tobacco, sugar beet,
        cotton, maize, wheat, barley, rice, sorghum, tomato, mango, peach, apple, mangel
        worzel, pear, strawberry, banana, melon, potato, carrot, lettuce, cabbage, onion, soya
        spp, sugar cane, pea, field beans, poplar, grape, citrus, alfalfa, rye, oats, turf and
        forage grasses, flax and oilseed rape, and nut producing plants insofar as they are not
        already specifically mentioned, their progeny, seeds and parts.

49.   Soybean, canola, brassica, cotton, sugar beet, sunflower, peas, potatoes and mangel
        worzels according to any one of claims 44-48.

50.   A method of selectively controlling weeds in a field, the field comprising weeds and
        plants or progeny according to any one of claims 44-49, the method comprising
        application to the field of a glyphosate type herbicide in an amount sufficient to
        control the weeds without substantially affecting the plants.

51.   A method according to the preceding claim, further comprising application to the
        field either before or after application of the glyphosate herbicide of one or more of

the following: a herbicide, insecticide, fungicide, nematicide, bacteriocide and an anti-viral.

52. A method of producing plants which are substantially tolerant or substantially resistant to glyphosate herbicide, comprising the steps of:

(i) transforming plant material with the polynucleotide of any one of claims 5 to 40 or the vector of claim 41;

(ii) selecting the thus transformed material; and

(iii) regenerating the thus selected material into morphologically normal fertile whole plants.

53. A method according to the preceding claim, wherein the transformation involves the introduction of the polynucleotide into the material by: (i) biolistic bombardment of the material with particles coated with the polynucleotide; or (ii) impalement of the material on silicon carbide fibres which are coated with a solution comprising the polynucleotide; or (iii) introduction of the polynucleotide or vector into *Agrobacterium* and co-cultivation of the thus transformed *Agrobacterium* with plant material which is thereby transformed and is subsequently regenerated.

54. A method according to the preceding claim, wherein the transformed material is selected by its resistance to glyphosate.

55. Use of the polynucleotide of any one of claims 5 to 40, or the vector of claim 41, in the production of plant tissues and/or morphologically normal fertile whole plants which are substantially tolerant or substantially resistant to glyphosate herbicide.

56. Use of the polynucleotide of any one of claims 5 to 40, or the vector of claim 41, in the production of a herbicidal target for the high throughput *in vitro* screening of potential herbicides.

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57.   A method of selecting biological material transformed so as to express a gene of
      interest, wherein the transformed material comprises the polynucleotide of any one of
      claims 5 to 40 or the vector of claim 41 and wherein the selection comprises exposing
      the transformed material to glyphosate or a salt thereof, and selecting surviving
5   material.

58.   A method according to the preceding claim, wherein the biological material is of
      plant origin.

10   59.   A method according to the preceding claim, wherein the plant is a dicot.

60.   A method according to the preceding claim, wherein the dicot is selected from group
      .consisting of soybean, cotton, sugar beet, *Brassica napus, campestris* or *oleraceae*.

15   61.   A method for regenerating a fertile transformed plant to contain foreign DNA
      comprising the steps of:

      (a)   producing regenerable tissue from said plant to be transformed;

      (b)   transforming said regenerable tissue with said foreign DNA, wherein said
      foreign DNA comprises a selectable DNA sequence, wherein said sequence functions
20   in a regenerable tissue as a selection device;

      (c)   between about one day to about 60 days after step (b), placing said
      regenerable tissue from step (b) in a medium capable of producing shoots from said
      tissue, wherein said medium may further contain a compound used to select
      regenerable tissue containing said selectable DNA sequence to allow identification or
25   selection of the transformed regenerated tissue;

      (d)   after at least one shoot has formed from the selected tissue of step (c)
      transferring said shoot to a second medium capable of producing roots from said
      shoot to produce a plantlet, wherein the second medium optionally contains the said
      compound; and

30   (e)   growing said plantlet into a fertile transgenic plant wherein the foreign DNA
      is transmitted to progeny plants in Mendelian fashion, wherein between step (b) and

step (c) there is an optional step of placing the transformed material onto callus inducing medium, characterised in that the foreign DNA is, or the selectable DNA sequence comprised by the foreign DNA comprises, the polynucleotide according to any one of claims 5 to 40, and the said compound is glyphosate or a salt thereof.

62.   A method according to the preceding claim, wherein the plant is a dicot selected from the group consisting soybean, cotton, sugar beet, *Brassica napus*, *campestris* or *oleraceae*.

63.   A method according to either of claims 61 or 62, wherein the said regenerable tissue is selected from the group consisting of embryogenic calli, somatic embryos, immature embryos etc.

64.   A diagnostic kit comprising means for detecting the enzyme of any one of claims 1-4 or that encoded by the polynucleotide of any one of claims 5 to 40 or the polynucleotide or vector of any one of claims 5 to 41.

1/6

Fig. 1

2/6
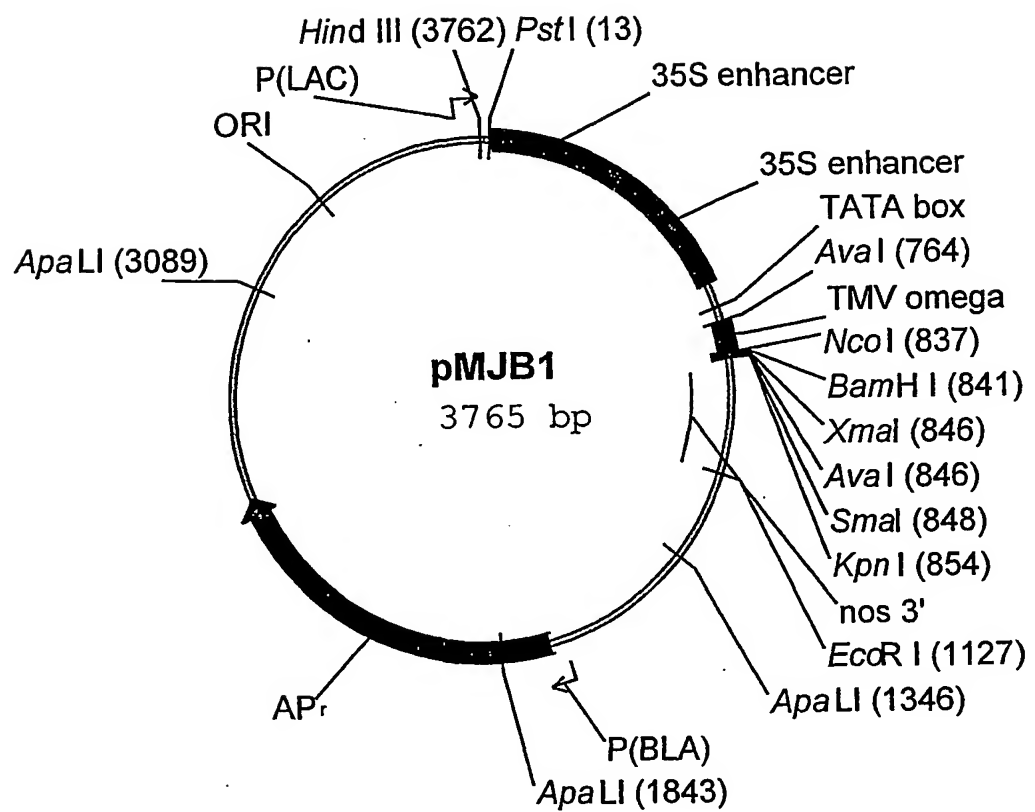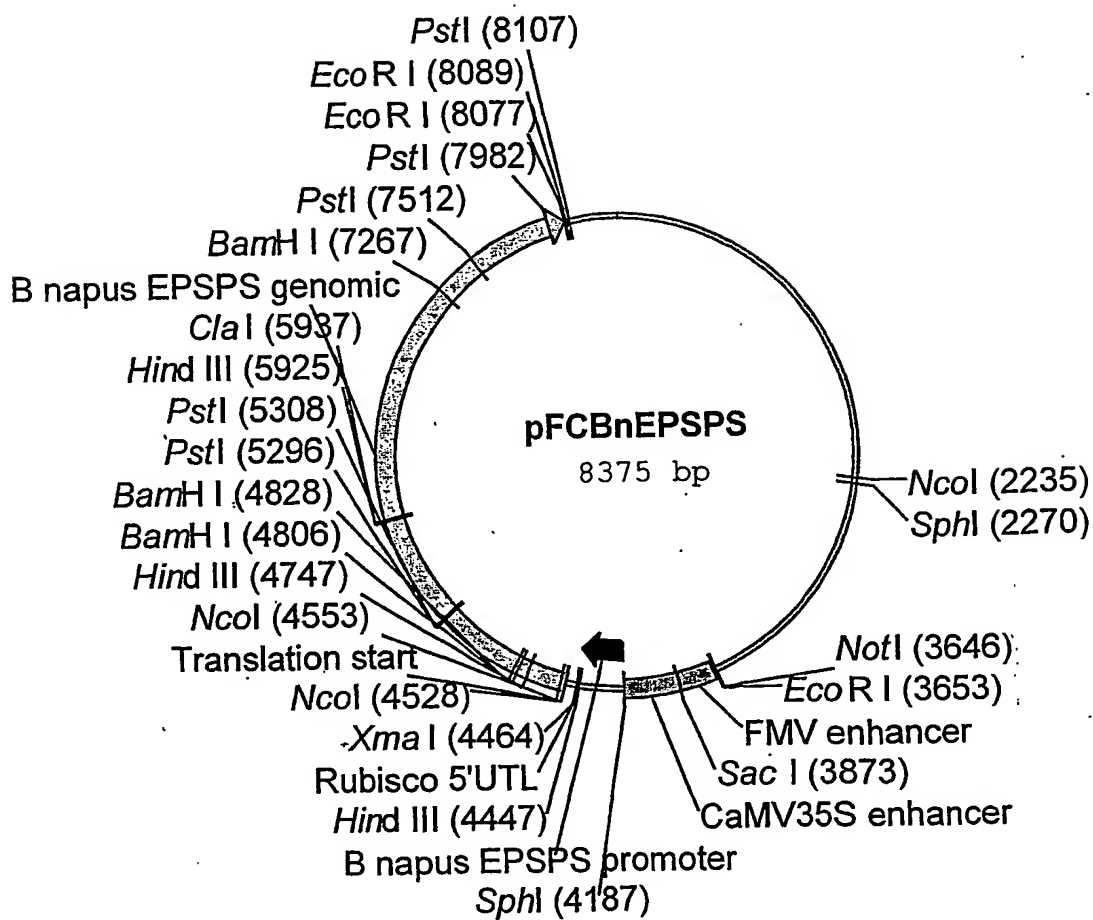
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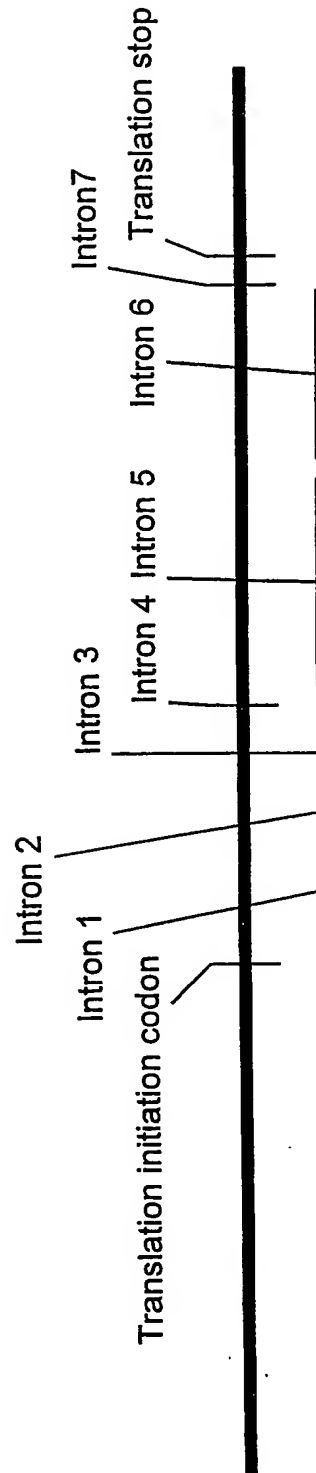
Soybean EPSPS 12

Fig. 3
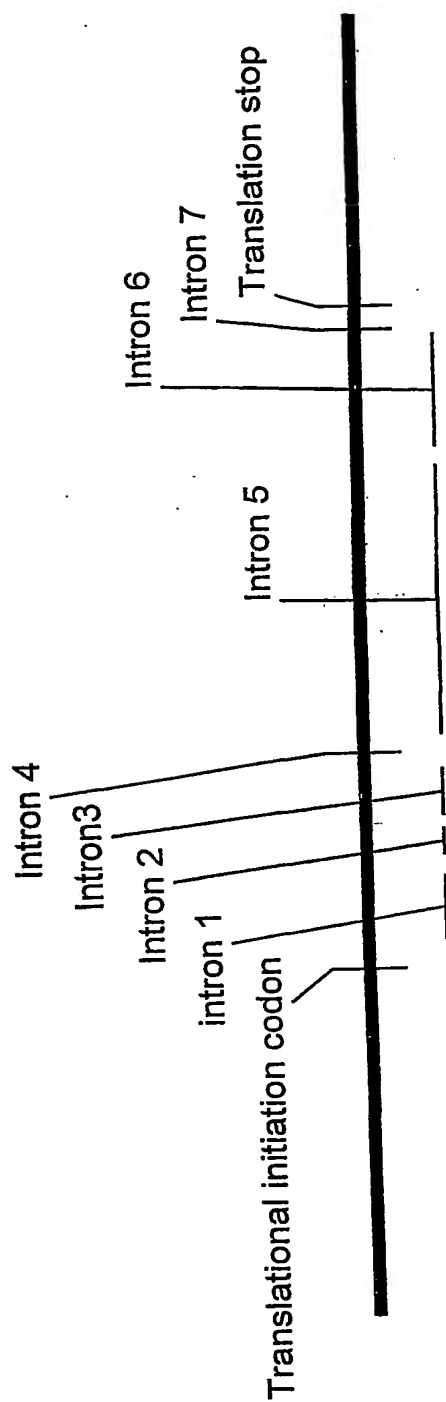
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Fig. 4

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ORI

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P(BLA)

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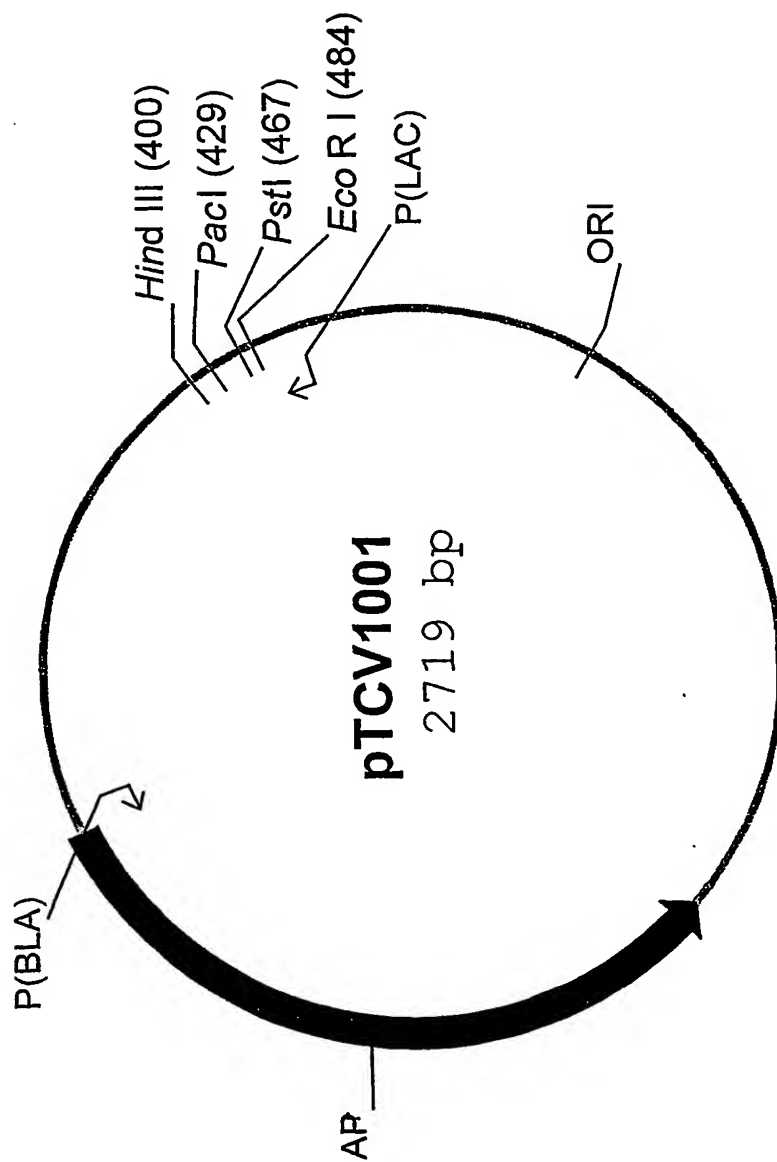
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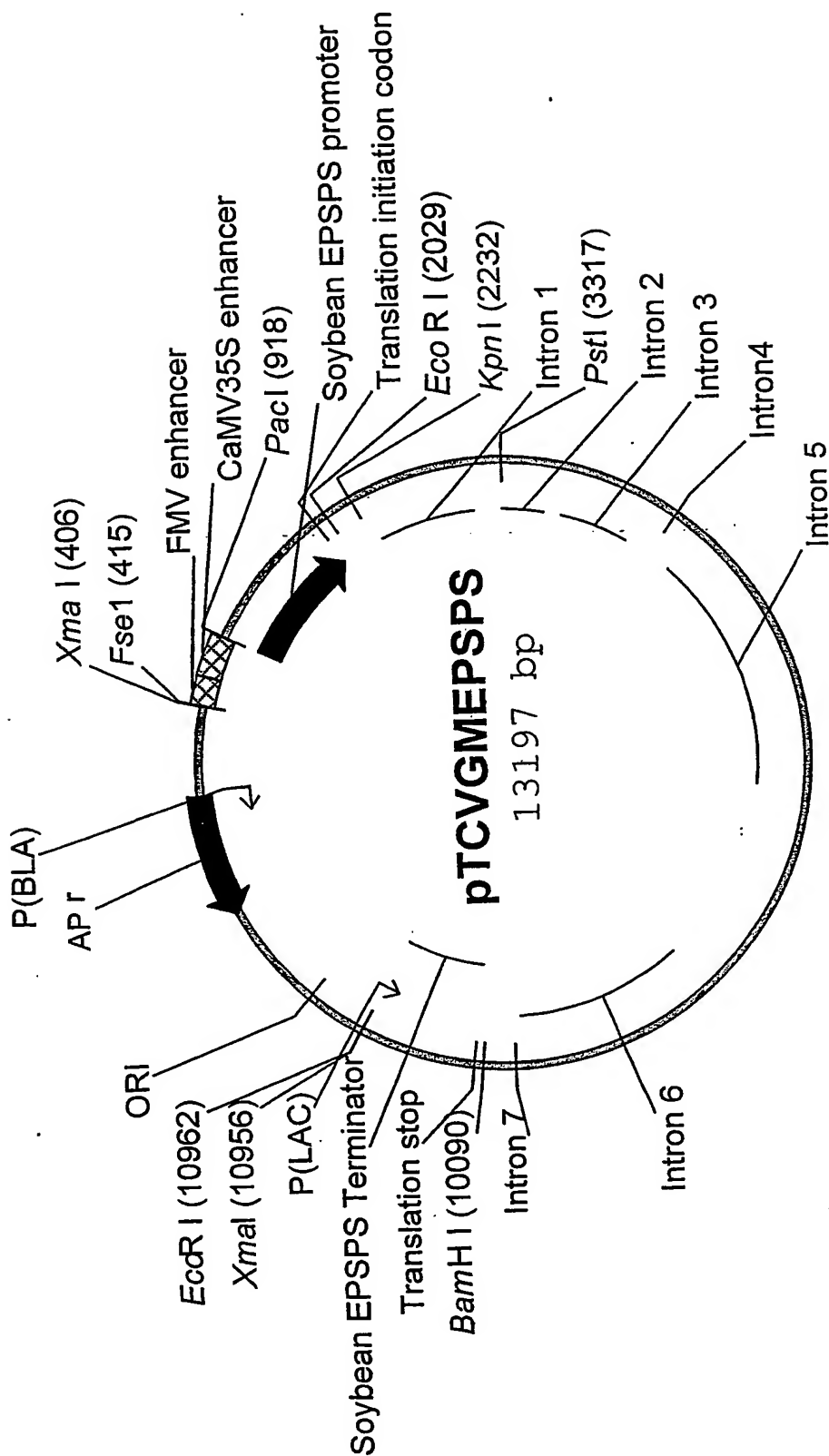
Fig. 6

SEQUENCE LISTING

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46

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ctctagtaca agtggggaac aaaataacgt ggaaaagagc tgtcctgaca gcccactcac  180

taatgcgtat gacgaacgca gtgacgacca caaaactcga gactttcaa caaagggtaa  240

tatccggaaa cctcctcgga ttccattgcc cagctatctg tcactttatt gtgaagatag  300

tggaaaagga aggtggctcc tacaaatgcc atcattgcga taaaggaaag gccatcgttg  360

aagatgcctc tgccgacagt ggtcccaaag atggaccccc acccacgagg agcatcgtgg  420

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taatgcgtat gacgaacgca gtgacgacca caaaactcga gaacatggtg gagcacgaca   240

cacttgtcta ctccaagaat atcaaagata cagtctcaga agaccagagg gctattgaga   300

cttttcaaca aagggtaata tcgggaaacc tcctcggatt ccattgccca gctatctgtc   360

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Val Glu

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   C12N15/54   C12N15/82   C12N9/10   A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 04114 A (CHAUBET NICOLE ;DEROSE RICHARD (FR); GIGOT CLAUDE (FR); RHONE POUL) 6 February 1997 (1997-02-06) page 12, line 20	1-64
Y	FR 2 736 926 A (RHONE POULENC AGROCHIMIE) 24 January 1997 (1997-01-24) page 2, line 11-16; claim 3	1-64
Y	DATABASE EMBL 'Online! Accession number X51475, 17 April 1990 (1990-04-17) GASSER CS, KLEE HJ: "Brassica napus 5-enolpyruvylshikimate-3-phosphate synthase gene" XP002188779 abstract	1-64

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[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2002	25/02/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Bilang, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PADGETTE S R ET AL: "SITE-DIRECTED MUTAGENESIS OF A CONSERVED REGION OF THE 5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE ACTIVE SITE" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 266, no. 33, 25 November 1991 (1991-11-25), pages 22364-22369, XP002023053 ISSN: 0021-9258 the whole document	
P,X	WO 00 66746 A (HAWKES TIMOTHY ROBERT ;PICKERILL ANDREW PAUL (GB); ZENECA LTD (GB)) 9 November 2000 (2000-11-09) the whole document	1-64
P,X	WO 01 24615 A (VALIGEN US INC) 12 April 2001 (2001-04-12) the whole document	1-64

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9704114	A	06-02-1997	FR	2736929 A1	24-01-1997
			AU	718521 B2	13-04-2000
			AU	6618496 A	18-02-1997
			BR	9609774 A	26-01-1999
			CA	2223881 A1	06-02-1997
			EP	0850311 A2	01-07-1998
			WO	9704114 A2	06-02-1997
			JP	11509414 T	24-08-1999
			US	6338961 B1	15-01-2002
			ZA	9606077 A	13-08-1997
FR 2736926	A	24-01-1997	FR	2736926 A1	24-01-1997
			AP	886 A	03-11-2000
			AU	6619196 A	18-02-1997
			BG	102247 A	30-04-1999
			BR	9609792 A	16-03-1999
			CA	2223875 A1	06-02-1997
			CN	1196088 A	14-10-1998
			CZ	9800174 A3	17-06-1998
			EA	980138 A1	29-10-1998
			EP	0837944 A2	29-04-1998
			WO	9704103 A2	06-02-1997
			HU	9900463 A2	28-05-1999
			JP	11510043 T	07-09-1999
			PL	324572 A1	08-06-1998
			SK	6498 A3	04-11-1998
			TR	9800065 T1	21-05-1998
WO 0066746	A	09-11-2000	AU	4133100 A	17-11-2000
			EP	1173580 A1	23-01-2002
			WO	0066746 A1	09-11-2000
			AU	4133900 A	17-11-2000
			BR	0010069 A	22-01-2002
			EP	1173581 A1	23-01-2002
			WO	0066747 A1	09-11-2000
WO 0124615	A	12-04-2001	AU	8005200 A	10-05-2001
			WO	0124615 A1	12-04-2001